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CONTENTS

Harry Baker Humphrey, 1873–1955 — Howard P. Barss 247
The influence of amino acids on the growth of two strains of Helminthosporium gramineum — W. P. Skoropad and D. C. Arny 249
Chlorogenic acid and other orthodihydricphenols in scab-resistant Russet Burbank and scab-susceptible Triumph potato tubers of different maturities — Gestur Johnson and Lawrence A. Schaal 253
Rhizoctonia disease of cotton in presence or absence of the cotton root-knot nematode in Arizona — Harold W. Reynolds and R. G. Hanson 256
Effect of amino acids on the virulence of Helminthosporium sativum to wheat seedlings — S. B. Hrushovetz 261
Grain storage studies XXIII. Time of invasion of wheat seed by various species of Aspergillus responsible for deterioration of stored grain, and source of inoculum of these fungi — John F. Tuite and Clyde M. Christensen 265
Badger Ballhead, a new cabbage resistant to yellows and mosaic — J. C. Walker, R. H. Larson, and G. S. Pound 269
Occurrence of mixtures of tristeza virus strains in citrus — Theodore J. Grant and Richard P. Higgins 272
Decrease in glycolic acid oxidase activity of wheat leaves infected with Puccinia graminis var. tritici — Z. Keraly and G. L. Farkas 277
Chronic decline, a tristeza-like bud-union disorder of orange trees — Henry Schneider 279
Reduction of the root rot complex on Croft lilies by fungicidal treatment and propagation from bulb scales — J. G. Bald and Philip A. Chandler 285
The systemic activity of cycloheximide in wheat seedlings — V. R. Wallen and R. L. Millar 291
Host range of a species of Trichodorus and its host-parasite relationships on tomato — R. A. Rohde and W. R. Jenkins 295
A leaf spot of Philodendron related to stomatal exudation and to temperature — Donald E. Munnecke and Philip A. Chandler 299
Pectolytic enzymes produced by Sclerotinia sclerotiorum — E. Echandi and J. C. Walker 303

Phytopathological Notes:

A modification of Bald's stain for viruses and for cell inclusions associated with virus infections — T. E. Rawlins 307
Mosaic and shoestring, virus diseases of cultivated blueberry in New Jersey — E. H. Varney 307
Stem lesions associated with Xanthomonas alfalfae — Raymond D. Brigham 309
Tomato rosette caused by a virus complex — G. R. Doering, W. C. Price, and S. B. Fenne 310
Report and abstracts of the 1957 annual meeting of the Southern Division of the American Phytopathological Society 312
Listing of books 314
Report of the 48th annual meeting of the American Phytopathological Society 315

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HARRY BAKER HUMPHREY, 1873–1955

Howard P. Barss

Harry Baker Humphrey, Vice President (1926), President (1945), Councilor for many years, and Life Member of the American Phytopathological Society, and Editor-in-Chief of PHYTOPATHOLOGY (1929-1943) passed from this life October 13, 1955, at the age of 82, in Los Altos, California.

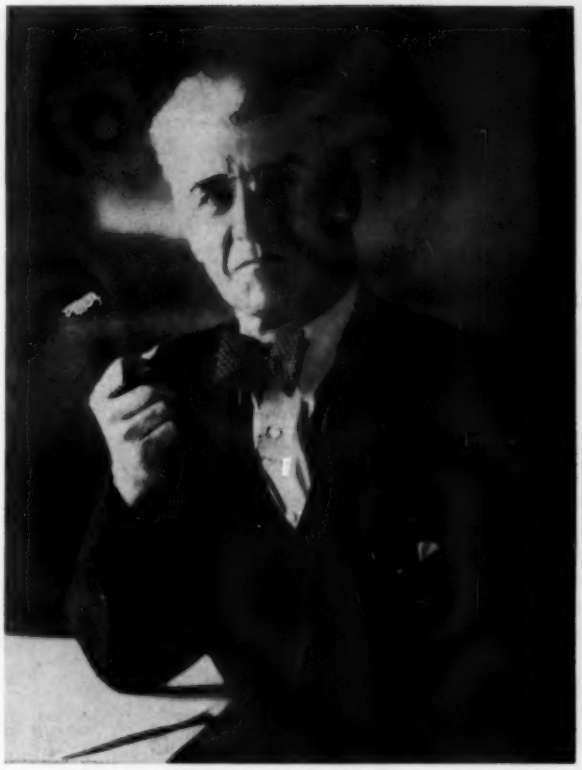
He was one of those able and enthusiastic members to whom the Society owes special gratitude. During its early years, he devoted unlimited time and energy in helping to put the Society and its Journal on a sound foundation and in establishing high standards for them both. His only compensation was the satisfaction of giving his best efforts to a purpose in which he believed.

Dr. Humphrey gave the Society 15 years of outstanding service as Editor-in-Chief and Councilor. During this period he was also Editor of Phytopathological Classics, for which his broad command of languages was a great asset. His 190-page translation from the French of Tillet's dissertation of 1755 on the smutting of wheat appeared in 1937 as No. 5 in this series. In his editorial work, his understanding and considerate helpfulness to inexperienced contributors and his patient insistence on correct and lucid English won high commendation for him and placed the Journal in the first rank among publications of its kind.

Dr. Humphrey pursued his major profession as a plant pathologist for 30 years in the U. S. Department of Agriculture. He and his co-workers, in cooperation with State Agricultural Experiment Stations, carried out important basic research on diseases of cereal grains and made significant progress in their practical control, particularly by testing and breeding for resistance.

Those who knew him best emphasize the strong constructive influence Dr. Humphrey exerted through his personal relationships during his long career. Seeking nothing for himself but the chance to be of service, he was a constant source of sympathetic encouragement, inspiration, and sound advice. Time and again his helpful words and understanding letters to troubled or discouraged workers gave new energy and direction to their endeavors.

Dr. Humphrey's staunch and vigorous personality was marked by absolute integrity, devotion to duty, unselfishness, and great kindliness. He was a versatile scholar with exceptionally wide interests; an able, stimulating, exacting teacher; a considerate and conscientious administrator; a linguist of parts; and a scientist of inquiring mind who stressed both fundamental research and its practical applications. He loved Nature in all her aspects and was attracted to the best in art, music, and literature. Above all, he possessed a dynamic faith in young people and a deep affection for his fellow men regardless of race, creed,



HARRY BAKER HUMPHREY, 1873–1955
Taken at about the age of 70, with editorial pen in hand.

or station. A man of quiet dignity, uncompromising toward slothfulness or low standards in conduct or language, Dr. Humphrey was conscientiously thorough and precise in every undertaking. Withal, he had great zest for life and a keen sense of humor revealed by the sparkle in his eyes and his energetic chuckle. He often regaled friends with inimitable tales in French-Canadian or in Swedish dialect.

Born August 4, 1873, in a log cabin on a homestead farm near the Minnesota frontier settlement of East Granite Falls, Harry was the first of the 5 children of John Wadsworth Humphrey and Adeline Regester Humphrey, descendants of respected early American colonists.

In 1877 the family moved to Oregon but 5 years later returned to farming in Minnesota near Elk River. Harry's first interest in plant life was awakened by his teacher in the nearby country school. At 18, encouraged by his mother, he decided that he must have further education even though the farm needed him. To get across the Mississippi to high school he built a row boat. Lumberjacks broke the lock and sent the boat downstream. Another boat was built only to be carried away in a log jam. Not

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to be stopped, he built a third. Doing a man's work on the home farm much of the time and rowing or skating over the river to his classes, Harry succeeded in graduating in 3 years from Elk River High School, president of his class.

Entering the University of Minnesota in 1895, he worked his way through, specializing in botany and modern languages. Through Dr. D. T. McDougal, renowned plant physiologist, he received an assistantship in botany at the University where he graduated with a B. S. degree in 1899. There followed 4 years of secondary school administration and teaching. In 1901 he married Olive Agatha Mealey of Minneapolis; she had been a fellow student in botany in the University. Through the years that followed, her judgement, understanding, and steadying influence were a great help to him and to their 6 children.

In 1903 he enrolled for graduate work in Leland Stanford Jr. University, where he become a part-time instructor and in 1907 received his Ph.D. degree. There D. H. Campbell and David Starr Jordan provided his chief inspiration. For 3 summers he taught marine biology at Hopkins Marine Biological Laboratory at Pacific Grove.

In 1908 Dr. Humphrey was appointed Professor of Botany and Plant Pathologist in the Experiment Station at the State College of Washington at Pullman. His successful work led to his promotion to Head of the Department of Botany and Vice Director of the Experiment Station in 1911. In the summer of 1912 he taught at the Marine Biological Laboratory at Friday Harbor on Puget Sound.

In 1913 he was appointed Pathologist in charge of cereal disease investigations in the Bureau of Plant Industry in the U. S. Department of Agriculture in Washington, D. C. Here he achieved the rank of Principal Pathologist in the Division of Cereal Crops and Diseases where he served until his automatic retirement in 1943, at the age of 70.

Dr. Humphrey's contributions to scientific literature numbered more than 50. His earliest works were concerned with algae, hepatics, and plant ecology. His later contributions, many with joint authorship, dealt mainly with cereal diseases, particularly the smuts and rusts. Although administrative work occupied much of his time, whenever possible he participated personally in the research he directed. In 1918 the important cooperative barberry eradication campaign in the North-Central States was initiated under his direction. Likewise, a nation-wide campaign for seed treatment against grain smuts was organized about that time. His studies with Woolman on the physiology of stinking smut in relation to infection and control and the studies of stripe rust of cereals (caused by *Puccinia glumarum*) conducted with C. W. Hungerford and A. G. Johnson are especially noteworthy. His effective cooperation with plant breeders in the Department of Agriculture and in State Experiment Stations was a great aid in their development of the disease-resistant lines of cereal grains, which have saved American agriculture from destructive losses. Dr. Humphrey extended helpful cooperation to his Canadian colleagues and was invited to address the Royal Society of Canada in Quebec in 1934.

From 1922 to 1948, after working hours, he taught French in the Department of Agriculture Graduate School; as a member of its Council and chairman of the Department of Languages and Literature, he was profoundly instrumental in its growth from an enrollment of only a few hundred students to more than 8,000. Many of his students of French went into foreign service.

Dr. Humphrey was deeply interested in the French-Canadian people, whom he felt were too little understood. His great affection for them rose from his boyhood contacts with French-Canadian neighbors in Minnesota. He visited Canada many times and finally wrote an account, as yet unpublished, of the French-Canadians, their customs and culture.

The family home, built with rammed-earth walls on Cabin John Run near Washington, D. C., in 1923, aroused much public attention as the first house of this type erected in this country in modern times. Dr. Humphrey first spent months of study on this rare kind of construction, tracing it back to the Romans of Pliny's time. He later collaborated with E. W. Coffin in publishing a *Handbook on Building Walls with Rammed Earth*.

Besides the American Phytopathological Society, Dr. Humphrey was a member of Sigma Xi, A.A.A.S. (Fellow), American Academy of Arts and Sciences, Washington Academy of Sciences, Botanical Society of America, Botanical Society of Washington (President 1930), Biological Society of Washington, Minnesota Historical Society, Cosmos Club, Washington College of Music, and American Federation of Arts. He was also active in church work.

In 1951, Dr. Humphrey and his wife moved to Los Altos, California, not far from Stanford University where he had access to the library in connection with the work which occupied his last years. At the time of his passing, he was working on a biography of North American botanists; he had completed the manuscript for about 85. He is survived by his wife, their 4 sons and 2 daughters, 16 grandchildren and 1 great grandchild. The 2 eldest sons and 1 daughter followed their father in the plant science field, Llewellyn M. as a geneticist and plant breeder, Robert R. as a plant ecologist and range conservationist, and I. Estella (Mrs. G. H. Godfrey) as a cytologist.

Our Society was fortunate in having the devoted service of such a man for so long a period. No less fortunate were the young men and women whom he encouraged to make the best of themselves and of their educational opportunities and those to whom he held out a helping hand in time of need. Dr. Humphrey will long be remembered as a true gentleman and scholar and as a great soul beloved because he lived "not to be ministered to but to minister."

THE INFLUENCE OF AMINO ACIDS ON THE GROWTH OF TWO STRAINS OF HELMINTHOSPORIUM GRAMINEUM [1]

W. P. Skoropad [2] and D. C. Arny [3]

SUMMARY

Two strains of *Helminthosporium gramineum* Rabh., which showed marked differences in pathogenicity on certain barley varieties, were grown on liquid media containing various amino acids and amides either singly or in combinations. The substances studied were those found in the free state in malt made from Oderbrucker barley. When the amino acids were used singly, fungus growth was greater than, less than, or the same as that in their absence, depending on the amino acid and fungus strain used. Responses to combinations of several amino acids indicated that certain ones, even though present in relatively small amounts, could exert considerable influence on the growth of the fungus strains.

Barley stripe, caused by *Helminthosporium gramineum* Rabh., is a systemic disease. The fungus penetrates the young seedlings through the coleorhiza and develops in the barley plant by spreading intercellularly and intracellularly in parenchymatous tissues as well as through the xylem vessels.

Histologic studies on susceptible and resistant reactions involving 2 pathogenic strains and 4 barley varieties were reported earlier (12). The resistant reaction resembled a type of hypersensitivity whereby the growth of the fungus was arrested soon after penetration. Since resistance appeared to be physiologic, it possibly could be explained in terms of the nutrition of the pathogen and the biochemistry of the host.

The vitamin, carbon, and nitrogen requirements of *H. gramineum* have been investigated. Elliott (4) found the fungus to be self-sufficient for vitamins of the B-complex. Converse (3) confirmed these results and also noted that the organism utilized the common carbon sources, glucose and sucrose, equally well in certain media; in combination with amino acids, however, sucrose gave better growth than glucose did. He also showed that *H. gramineum* utilized the nitrogen in nitrate, in ammonia, or in amino form. Marked differences were noted in growth response to single amino acids when used with an optimum supply of inorganic nitrogen from ammonium nitrate. This finding prompted Converse to suggest a possible relationship between the growth response of *H. gramineum* in culture to amino acids and the establishment of the fungus in the host.

The kinds and quantity of free amino acids reported as being present in barley plants have varied considerably (2, 7, 13). These differences may be attributed to the age and variety of the plants or to the methods of extraction and analysis. To our knowledge, no work has been done on varietal differences.

The objective of this study was to determine the growth responses in culture of 2 strains of *H. gramineum* to the free amino acids that are known to be present in young barley seedlings.

MATERIALS AND METHODS.—*Fungus strains.*—The 2 strains of *H. gramineum*, designated as C-1 and GHA2, have been described earlier (1, 11). Both strains had been carried in culture on potato-dextrose agar by mass transfers for a number of years and had been stable in cultural characters and in pathogenicity. The barley variety Atlas (C. I. 4118) [4] was highly susceptible to strain GHA2 and highly resistant to strain C-1. Oderbrucker (C. I. 4666) was highly susceptible to C-1 and highly resistant to GHA2. Colsess (C. I. 5979) was highly susceptible to both strains, whereas selection B39-55 (from a cross of Atlas and Oderbrucker) was highly resistant to both strains.

Amino acids used.—The choice of amino acids used in these studies was based on preliminary analyses of 6-day malt from Oderbrucker barley. In the analyses, made by the U. S. Barley and Malt Laboratory, Madison, 19 free amino acids and amides were found. Sixteen of these were used in studies in response to single acids, and all were used in the medium designed to simulate the free amino acid content of Oderbrucker malt.

Cultural methods and growth measurement.—The growth response of C-1 and of GHA2 to nutrient solutions was determined by weighing the mycelium produced on 50 ml of liquid medium in 250-ml Erlenmeyer flasks. The method of Converse (3) was used.

Inoculum discs 3 mm in diameter were cut from the edges of a 5-day-old colony on potato-dextrose agar. A disc was placed aseptically on the bent end of a glass rod that passed through the cotton plug of an Erlenmeyer flask. The rod was then lowered so that it was in contact with the nutrient solution. The flasks were placed in the dark at 24± 1°C, the optimum temperature reported by Shands (10). After 14 days, the compact mycelial mat was washed repeatedly, air-dried for 16 hours in a 95° oven, and weighed. Aver-

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[3] Professor of Agronomy and Plant Pathology, University of Wisconsin, Madison, Wisconsin.

[4] C. I. refers to accession number of Cereal Crops Section, Agricultural Research Service, U. S. Department of Agriculture.

age weights were based on the mycelium from 5 flasks. The pH of the medium was determined with a Beckman pH meter before and after incubation.

Basal medium.—The basal medium was the same as that used by Converse (3), except that sucrose was substituted for glucose as the carbon source because of an adverse interaction between amino acids and glucose during autoclaving. The basal medium was prepared as follows: sucrose, 38.0 g; NH_4NO_3, 0.7 g (0.245 g nitrogen/1); KH_2PO_4, 0.3 g; K_2HPO_4, 1.2 g; $MgSO_4 \cdot 7H_2O$, 0.3 g; $FeCl_3 \cdot 6H_2O$, 1 ppm; $ZnSO_4 \cdot 7H_2O$, 1 ppm; $CuSO_4 \cdot 5H_2O$, 1 ppm; $MnSO_4 \cdot 5H_2O$, 1 ppm; and distilled water, 1 liter.

EXPERIMENTAL RESULTS.—*Optimum ammonium nitrate concentration.*—Neither fungus strain showed a sharp optimal point, but instead best growth occurred over a range centering around 0.122 g nitrogen per liter. An increase in the amount to 0.245 g/1 did not have a statistically significant effect on fungus growth. Thus the 0.122 g/1 was considered optimal. In the basal medium, twice the optimal amount was used so that the nitrogen concentration was uniform in basal and amino acid media.

Growth response of each fungus strain on the basal medium was tested in 5 different experiments. Variation in growth within and among experiments was not statistically significant. The average weight of dry mycelium was 642 mg for C-1 and 403 mg for GHA2.

Optimum pH and pH range.—Both strains grew on basal media ranging in pH from 3.0 to 9.5. Strain C-1 grew best at pH 5.6 and GHA2 at pH 6.8; however, there was no sharp optimal pH for either strain.

Growth response to single amino acids.—In the first group of experiments, 15 amino acids and 2 amides were added singly to the basal medium. The total nitrogen, 0.245 g/1, was furnished in equal amounts by ammonium nitrate and by the amino acid. Since this gave an optimum supply of inorganic nitrogen, it was assumed that any effect on the growth of the fungus could be attributed to the properties of the amino acid rather than to an increase in nitrogen supply.

The data on growth of C-1 and of GHA2 on 15 amino acids and 2 amides are shown graphically in Figure 1. The effect of these nitrogen sources on the growth of each strain was calculated as the percentage increase or decrease in weight of mycelium in the amino acid medium as compared to the average weight attained in the basal medium. This provided a basis for comparing growth of strain C-1 with that of strain GHA2.

Growth tests on leucine, glycine, valine, asparagine, glutamic acid, isoleucine, aspartic acid, or phenylalanine were repeated; there was no statistically significant difference between tests in growth on any one amino acid.

The results presented in Figure 1 demonstrated 3 general facts: 1) each strain of *H. gramineum* showed a great diversity of growth response to the amino acids; 2) there was a marked difference in growth of the 2 strains on any 1 amino acid; and 3) growth of strain GHA2 was increased considerably more than that of strain C-1.

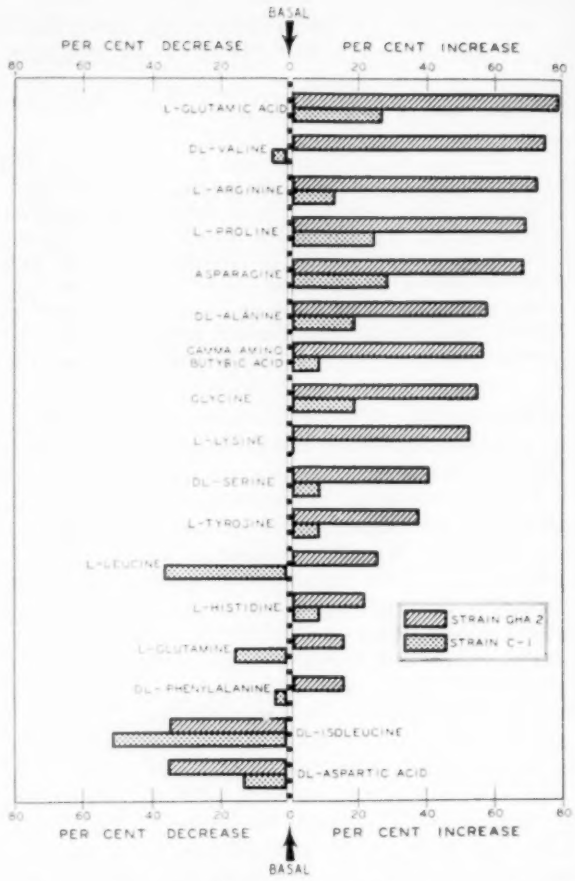

FIG. 1. Relative influence of several amino acids and amides on the growth of 2 strains of *H. gramineum* in liquid culture. Optimum nitrogen was supplied by NH_4NO_3. For purposes of comparison, measurements were made in terms of dry weight of mycelium and converted to percentage decrease or increase in growth in relation to growth on the basal medium.

The greatest effect on growth of GHA2 was by glutamic acid, which increased growth by 78 per cent over that in the basal medium. On the other extreme, both isoleucine and aspartic acid decreased growth of GHA2 by 35 per cent. Asparagine was most effective on the growth of C-1, but the increase over that on the basal medium was only 30 per cent. Isoleucine reduced growth of C-1 by 50 per cent, whereas valine, lysine, or phenylalanine had no marked effect on growth of this strain. Perhaps the most striking examples of the difference in growth response of the 2 strains were in media containing leucine or glutamine. In both instances, growth of GHA2 was increased whereas that of C-1 was decreased.

Aspartic acid decreased growth of both strains, whereas asparagine, which is the amide form of this acid, increased growth. However, both glutamic acid and its amide, glutamine, caused an increase in

growth.

The original pH of each medium, except those containing aspartic or glutamic acid, was close to neutrality. The pH of all media following 14 days' incubation ranged from 6.2 to 7.0 and from 6.3 to 6.8 for C-1 and GHA2, respectively. On aspartic acid or glutamic acid medium, both fungus strains grew as well on that initially adjusted to pH 6.7 as on unadjusted media (pH 4.5). This indicated that pH at these levels did not determine growth of the fungus on these 2 amino acids.

Growth response to combinations of 2 amino acids.—Growth response of C-1 and of GHA2 was tested also on several media each containing a combination of 2 amino acids in the basal medium. Ammonium nitrate furnished 0.122 g nitrogen per liter, and the 2 amino acids supplied a similar amount, each contributing an equal share.

When used singly, isoleucine or aspartic acid decreased growth of both strains, whereas glutamic acid or asparagine increased growth of both (Table 1). When isoleucine and glutamic acid or asparagine and aspartic acid were combined, the amount of growth was approximately the average of that obtained when the amino acids were used singly. When the 2 inhibitory amino acids were combined, growth again was intermediate for each strain.

Growth response to an association of amino acids.—It was assumed that there might be a difference in the way an amino acid influenced the growth of the fungus strains depending on its use singly or in association with other amino acids in the medium. This hypothesis was tested by experiments with a medium containing all the amino acids known to be present in 6-day malt from Oderbrucker barley. The salts and carbon sources were the same as in the basal medium, but the sole source of nitrogen was the amino acids. The complete medium included the amino acids in the relative proportions found in the seedlings and in amounts that supplied 0.245 g of nitrogen per liter.[5] Six of the amino acids were omitted one at a time, and any difference in growth of the fungus from its growth in the complete medium was attributed to the missing amino acid.

Since isoleucine when used singly in a medium strongly inhibited growth of both fungus strains (Fig. 1), less fungus growth would be expected in any medium containing isoleucine than in the same medium minus isoleucine. In the tests on media containing several amino acids, however, there was no statistically significant difference in weight of mycelium on the complete medium and in the medium lacking isoleucine (Table 2). This indicated that the inhibitory effect of isoleucine was neutralized when this amino acid was used in combination with other amino acids. Aspartic acid, which also was inhibitory to both strains when used singly, was inhibitory in the complete medium to C-1 but not to GHA2. Strain C-1 behaved as was expected with respect to glutamic acid and proline, since growth was reduced when they were omitted. Growth of strain GHA2 was increased by these 2 materials when they were used singly, but its growth was not reduced when they were omitted from the medium. Histidine, which increased growth of C-1 slightly when used singly, appeared to be inhibitory in the complete medium, since growth increased when histidine was left out.

The most interesting difference was found between the amount of fungus growth on the complete medium and that on the medium with leucine omitted. The latter medium gave significantly greater growth of C-1 and less growth of GHA2 when compared to the control medium. When used singly, leucine decreased growth of C-1 by 36 per cent and increased that of GHA2 by 24 per cent.

DISCUSSION.—The report of Converse (3) concern-

TABLE 1.—*Growth after 2 weeks of 2 strains of Helminthosporium gramineum on basal medium supplemented with amino acids, singly and in pairs*

Amino acids in medium[a]	Weight in mg of indicated strain	
	C-1	GHA2
Isoleucine + glutamic acid	473	552
Asparagine + aspartic acid	626	513
Isoleucine + aspartic acid	390	282
Isoleucine	318	263
Glutamic acid	817	717
Asparagine	832	673
Aspartic acid	575	375

[a] Basal medium contained sucrose and various salts, including NH_4NO_3 sufficient to provide 0.122 g N/l. The amino acids also supplied 0.122 g N/l, with each member of a pair supplying an equal amount.

TABLE 2.—*Growth after 2 weeks of 2 strains of Helminthosporium gramineum in combinations of amino acids and amides*

Medium[a]	Growth in mg of indicated strain	
	C-1	GHA2
Complete (19 amino acids and amides)	703	710
Glutamic acid omitted	657	713
Proline omitted	604	701
Isoleucine omitted	696	708
Leucine omitted	793	667
Aspartic acid omitted	746	705
Histidine omitted	794	697
LSD (5 per cent level)	27	17

[a] The complete medium contained sucrose and various salts in the proportions used in the basal medium. The sole source of nitrogen, 0.245 g/l, consisted of amino acids in the relative proportions found in 6-day malt from Oderbrucker barley. Wherever an amino acid was omitted, the others were increased to make up a total of 0.245 g N/l.

[5] The relative proportions of amino acids and amides in the control medium were as follows: asparagine, 1.40; serine, 1.40; threonine, 1.40; glutamic acid, 1.00; aspartic acid, 1.40; alanine, 1.10; arginine, 2.10; γ-amino butyric acid, 0.72; leucine, 0.64; tryptophane, 2.70; valine, 0.49; glycine, 0.39; isoleucine, 0.66; phenylalanine, 1.60; tyrosine, 1.00; lysine, 0.87; proline, 3.40; histidine, 1.30; and cystine, 0.24.

ing the diversity of growth responses of an isolate of *H. gramineum* to different amino acids was confirmed for strains C-1 and GHA2 used in the present study. Each of these strains showed not only great diversity of growth on various amino acids but also responded differently to any 1 amino acid. Differences in growth response of strains of fungus species to amino acids have been reported for *Helminthosporium sativum* Pam., King & Bakke by Peterson and Katznelson (9), for *Venturia inaequalis* (Cke.) Wint. by Leben and Keitt (6), and for *Phytophthora infestans* (Mont.) d By. by French (5).

An incubation period of 14 days and the composition of the basal medium were the same in all tests. The growth results obtained, therefore, applied under those conditions only. Pelletier and Keitt (8) demonstrated that growth responses of *V. inaequalis* to certain amino acids were affected considerably by the basal medium composition, the amino acid concentration, and the duration of the incubation period.

Since minor changes in the pH of the medium did not alter significantly the growth of either strain in the present experiments, and since the media contained an optimum amount of inorganic nitrogen, differences in the amount of growth possibly may be attributed to some property of the amino acid concerned. No correlation appeared to exist, however, between the structure of the amino acids and their relative effects on growth of either strain. It was possible that certain amino acids may have contained trace elements as contaminants and that increases in growth of the fungus were attributable to the added element(s) rather than to the amino acid. This theory may be ruled out in these experiments on the basis that some amino acids caused a decrease in growth of the fungus. Furthermore, certain ones, like glutamic acid, that increased growth when added singly to the basal medium did not have a similar effect when included in the basal medium in combination with an inhibitory amino acid such as isoleucine. If trace elements were present as contaminants, they were added in both instances.

Growth on a limited number of media containing combinations of 2 amino acids showed no dominant influence by either, but rather each one appeared to exert independently its effect on fungus growth. This resulted in growth that was approximately intermediate between the amounts obtained on media containing these amino acids singly.

Growth was satisfactory on media in which the sole source of nitrogen was from amino acids supplied in proportions present in Oderbrucker barley malt. Of the 6 amino acids that were omitted 1 at a time from the complete amino acid medium, only leucine appeared to have the same effect when used with other amino acids as when used alone. When used singly, it increased growth of GHA2 and decreased that of C-1, even when relatively small quantities of it were present in the control medium. The absence of leucine from the complete amino acid medium resulted in reduced growth of GHA2 and increased growth of C-1. In the other media from which single amino acids (except isoleucine) were omitted, growth of C-1 differed significantly from that in the medium containing all the amino acids. There was no apparent relationship, however, between the influence on growth of these amino acids when used singly and when used in association with other amino acids.

The 2 fungus strains respond differently to the various amino acids, regardless of whether these are used singly or in combinations of 2 or more. These growth responses in culture possibly may have similar expressions inside barley seedlings, because *H. gramineum* develops systemically and is in immediate contact with the nutrilites of the host. Although this variability in amino acid nutrition has not been tested for correlation with the pathogenicity of the 2 strains of the barley stripe fungus, it indicates that amino acid nutrition of the fungus is of potential importance in relation to this phenomenon.

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CHLOROGENIC ACID AND OTHER ORTHODIHYDRICPHENOLS IN SCAB-RESISTANT RUSSET BURBANK AND SCAB-SUSCEPTIBLE TRIUMPH POTATO TUBERS OF DIFFERENT MATURITIES[1]

Gestur Johnson and Lawrence A. Schaal

SUMMARY

The concentration of chlorogenic acid, as determined by quantitative paper chromatography, and that of total *o*-dihydricphenols, as determined by Arnow's colorimetric procedure, were found to be much higher in the periderm of tubers of the scab-resistant variety Russet Burbank than in that of tubers of the scab-susceptible variety Triumph. The greatest difference between the varieties was found at the stage where the tubers were growing most rapidly. Anaylsis of the tuber flesh for total *o*-dihydricphenols showed no consistent difference between the 2 varieties during the growing period. The flesh of both varieties was found to be very low in *o*-dihydricphenols as compared with that of the periderm. The tubers were analyzed at 3 stages of growth and after 5 months of storage at 35°–38° F. The results obtained indicate that the ferric chloride spot test for scab resistance should be made on immature or on freshly harvested mature tubers.

In previous publications[3,4] it was reported that the chlorogenic acid content of the periderm tissue of the potato tuber varied with the potato variety. The varieties high in chlorogenic acid were in general more resistant to scab caused by *Streptomyces scabies* (Thaxt.) Waks. & Henrici than were varieties low in this compound. The nature of the relation of chlorogenic acid to scab resistance has been discussed by the authors[5]. Since most infections by *S. scabies* are initiated when the tubers are small, it appeared desirable to determine the chlorogenic acid content of the periderm tissue of young tubers in resistant and susceptible varieties. The experiments described in this paper concern the relative amounts of chlorogenic acid in the periderm of the tubers of the resistant variety Russet Burbank and the susceptible variety Triumph. Tests were made on 1) immature, 2) mature, and 3) mature tubers stored for 5 months.

MATERIALS AND METHODS OF ANALYSIS.—Two varieties of potatoes (*Solanum tuberosum* L.), Triumph and Russet Burbank were used in this investigation. Each variety was sampled 1) when the tubers weighed approximately 20–40 g; 2) when the tubers were growing rapidly and weighed 90–160 g; 3) when the tubers were mature; and 4) after 5 months' storage at 35°–38° F. The total number of tubers used varied with the different sampling dates.

The tubers were peeled with a "Nee Action" vegetable peeler, which gave uniform peelings approximately 1 mm in thickness.

One hundred g of peelings was added to 300 g of 95 per cent ethanol and choped in a Waring Blendor for 5 minutes. The total volume of the ethanol extract, after correction for volume of pulp, was 453 ml. Approximately 250 ml of the extract was filtered through a Whatman No. 12 fluted filter into a 250-ml Erlenmeyer flask. The extract was then placed in freezing temperature storage (−20° F) until a convenient time for analysis.

Analysis of chlorogenic acid by paper chromatography.—A 25-ml aliquot of the ethanol extract was concentrated under reduced pressure to approximately 3 ml. It was then quantitatively transferred to a 10-ml centrifuge tube and made to volume by the addition of 95 per cent ethanol. A small amount of insoluble material was removed by centrifugation.

A 40-μl aliquot was laced on a Whatman No. 1 paper chromatogram (17 × 16 in.) by placing 20 applications of 2 μl each in the form of adjacent spots to form a band approximately 10 cm long. Two such bands were placed on each chromatogram. After the chromatographing in a 40:10:20 mixture of *n*-butanol, acetic acid, and water, the chlorogenic acid bands were located by observing the chromatogram under ultraviolet light. The chlorogenic acid band was cut from the chromatogram and eluted from the paper with 70 per cent ethanol. The eluate was made to 10 ml. The optical density of the eluate at 324 mμ was determined with a Beckman Model DU Spectrophotometer. The $E^{1\%}_{1cm}$ value of 526 for anhydrous chlorogenic acid as

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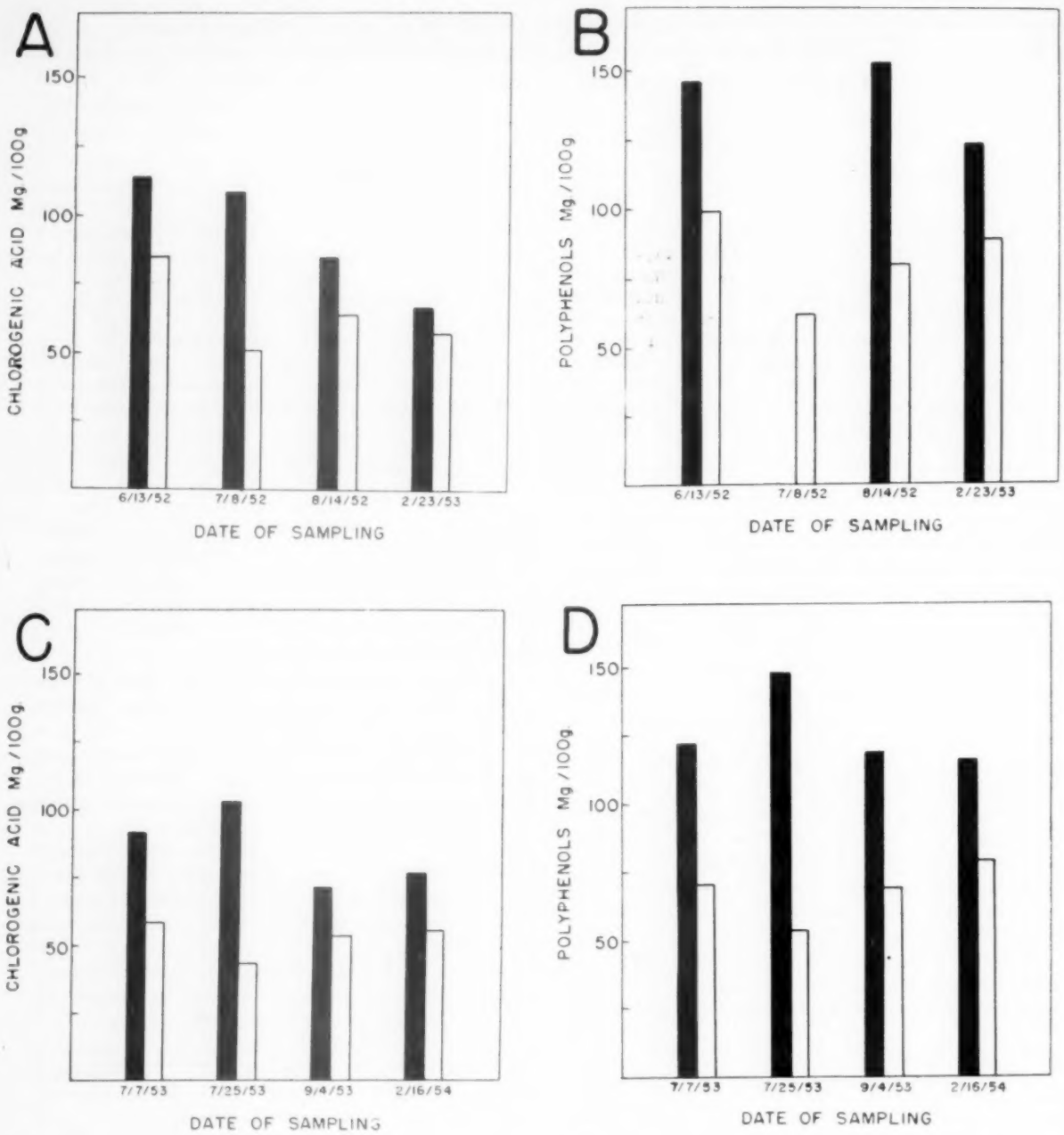

FIG. 1. Chlorogenic acid and total *o*-dihydricphenols in periderm tissue of tubers of the varieties Russet Burbank (solid bar) and Triumph (open bar) at various stages of maturity and after several months of storage. The 4 days of sampling were when tubers weighed 20–40 g, when tubers weighed 90–160 g, when tubers were mature, and after 5 months' storage at 35°–38° F, respectively. A) Chlorogenic acid content of 1952 crop as determined by paper chromatography. B) Total polyphenolic content of 1952 crop as determined by Arnow's reagent. C) Chlorogenic acid content of 1953 crop as determined by paper chromatography. D) Total polyphenolic content of 1953 crop as determined by Arnow's reagent.

reported by Moore et al[6] was used in the calculation of the amount of chlorogenic acid.

[6] Moore, R. G., D. L. McDermott, and A. T. Wood. Determination of chlorogenic acid in coffee. Anal. Chem. 20: 620-624.

The accuracy of the method described was tested by using a solution of known concentration of chlorogenic acid, 25 mg/50 ml 70 per cent ethanol. The percentage recovery was found to vary between 91.5 and 95. Thus the values for chlorogenic acid obtained by the paper chromatographic method appeared to be slightly

low.

Chlorogenic acid and other o-dihydricphenols by Arnow's reagent.—Arnow's reagent[7] is quite specific for *o*-dihydricphenols. This reagent not only will measure chlorogenic acid, the principal *o*-dihydricphenol in potatoes, but also other *o*-dihydricphenols that may be present in small concentrations.

One ml of the ethanol extract diluted to twice its volume with distilled water was pipetted into a Evelyn colorimeter tube to which were then added the following reagents: 1 ml 0.5 N HCl, 1 ml Arnow's reagent (10 g $NaNO_2$ and 10 g Na_2MoO_4 in 100 ml H_2O), 10 ml distilled water, and 2 ml NaOH. Thirty seconds after the addition of the alkali, the pink color produced was read in a Evelyn colorimeter equipped with a 515-mμ filter. In the blank sample used for the adjustment of the instrument reading to 100 per cent transmission, the Arnow's reagent was replaced by 1 ml of H_2O. The optical density of 0.1 mg anhydrous chlorogenic acid per 15 ml of the reaction mixture (optical density = 0.248) was used in the calculation of chlorogenic acid.

Analysis of potato flesh.—The total *o*-dihydricphenols (by Arnow's reagent) were determined also in the flesh of some of the potato samples. In these samples, care was taken that all traces of peelings were removed before the samples were extracted. The extraction procedure used was the same as that for peelings. One-ml aliquots of the undiluted ethanol extract were used.

RESULTS AND DISCUSSION.—*Analysis of potato peelings.*—The results obtained by the colorimetric method were consistently higher than those obtained by quantitative paper chromatography (Fig. 1). This was due to the facts that paper chromatography gave somewhat low values for chlorogenic acid and that the Arnow's reagent also measures other *o*-dihydricphenols that may be present.

Chlorogenic acid and other *o*-dihydricphenols in general were present in highest concentration in peelings during the early stages of tuber growth. The difference between the Russet Burbank and Triumph varieties was also greatest during the early stages of growth, and particularly during the stage when the tubers were growing most rapidly (second sampling). This observation may be very significant, since most infections by *S. scabies* are initiated at this stage of tuber development. The initial chlorogenic content of the Triumph variety apparently was not maintained during the period of most rapid tuber growth. Since this was found to be the case in 2 years during which the growing conditions differed greatly, it is doubtful that it was a chance occurrence.

TABLE 1.—*Amount of total o-dihydricphenols in the flesh of Triumph and Russet Burbank potato tubers*

Crop	Date of sampling	Variety	*o*-dihydricphenols[a] (mg/100 g)
1952	8-14-52	Burbank	9.1
		Triumph	10.3
1952	2-23-53	Burbank	10.6
		Triumph	8.6
1953	7- 7-53	Burbank	4.8
		Triumph	6.0
1953	7-25-53	Burbank	5.7
		Triumph	5.1
1953	9- 8-53	Burbank	6.2
		Triumph	6.2
1953	2-16-54	Burbank	7.1
		Triumph	8.4

[a] Determined by Arnow's reagent.

Because the difference between the 2 varieties became less after several months of storage, the ferric chloride spot test[4] as an indicator for resistance should be made only on young or freshly harvested mature tubers. It also should be emphasized that the $FeCl_3$ spot test, like the Arnow's reagent, measures not only chlorogenic acid but other *o*-dihydricphenols. It can be assumed that these polyphenols could also play a role in the scab-resistance mechanism. For this reason, the total *o*-dihydricphenolic content of the periderm may be a better measurement for resistance than is chlorogenic acid alone, although the latter is the principal polyphenolic component.

No attempt was made to identify the minor *o*-dihydricphenols. Traces of caffeic acid, however, were found in the extracts of the peelings from mature tubers and a small amount in extracts of the peeling from the stored tubers. No free caffeic acid was detected in the chromatograms of the extracts of the peelings from the young immature tubers.

Orthodihydricphenols in flesh of potato tubers.—There appeared to be no differences in the polyphenolic contents of the flesh of tubers of the 2 varieties (Table 1). The stage of growth also appeared to have no effect. The polyphenolic content of the flesh is very low as compared with that of the peelings.

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RHIZOCTONIA DISEASE OF COTTON IN PRESENCE OR ABSENCE OF THE COTTON ROOT-KNOT NEMATODE IN ARIZONA [1]

Harold W. Reynolds and R. G. Hanson [2]

SUMMARY

Field surveys in 1952 and 1953 to determine the cause of a cotton seedling disease in Arizona indicated that the presence of the cotton root-knot nematode, *Meloidogyne incognita acrita* Chitwood affects the incidence of postemergence damping-off of cotton, primarily caused by the fungus *Rhizoctonia solani* Kühn. Field surveys and experiments under controlled conditions in the screenhouse and field to determine the relationship between the nematode and the fungus in this disease showed that an increase in Rhizoctonia disease was associated with an increase in the incidence of root-knot nematodes and with a corresponding decrease in plant size and survival. In fumigated plots where nematodes were controlled, the loss in stand due to Rhizoctonia was less than in unfumigated plots. Root-knot nematodes as debilitating parasites affect plant vigor, thus weakening the plant and making it more susceptible to attack by Rhizoctonia. Experiments in which cotyledons were excised further demonstrated that factors affecting seedling vigor, such as mechanical injury, contribute to this type of damping-off.

During the springs of 1952 and 1953, field surveys were made to determine the disease problem in cotton seedlings, *Gossypium hirsutum* L., in Pinal and Maricopa counties, Arizona. The amount of damping-off varied from field to field, ranging from little or none to enough to cause considerable loss in stand. Numerous pure-culture isolates from diseased tissue of cotton seedlings demonstrated that the primary fungus associated with damping-off in all areas surveyed was *Rhizoctonia solani* Kühn. This type of damping-off, commonly called "soreshin," is referred to in this paper as Rhizoctonia disease of cotton.

The amount of Rhizoctonia disease seemed to be affected by a number of predisposing factors such as temperatures below optimum for rapid plant growth, land preparation and planting methods, insects, and soil-borne parasites. These factors affected the vigor of the host and appeared to increase susceptibility of the seedlings to Rhizoctonia.

Many sandy-textured fields in several of the cotton-growing areas are rather heavily infested with the cotton root-knot nematode, *Meloidogyne incognita acrita* Chitwood. Cotton seedlings in such infested fields appeared to be more severely stunted than those in fields which had few, if any, of this root-knot species, and more damping-off occurred. These observations suggested a nematode-Rhizoctonia complex. Accordingly, experiments were conducted in the screenhouse and in field plots to determine whether the nematode contributed to a greater incidence of the Rhizoctonia disease.

REVIEW OF LITERATURE.—The role of temperature as a factor in the incidence of Rhizoctonia disease of cotton was investigated by Walker (10) in 1928. In detailed soil-temperature studies, he found that although Rhizoctonia grew best in culture between 27° and 29° C, the optimum temperature range for it to kill cotton seedlings was between 17° and 23°.

Neal and Newsom (4) investigated the relation of thrips to Rhizoctonia disease of cotton seedlings. They demonstrated that when cotton seedlings are damaged by thrips, the leaf area is greatly reduced and that the incidence of Rhizoctonia disease is much higher.

Arndt and Christie (1) conducted experiments with 4 species of nematodes, 1 each from the genera *Aphelenchoides, Aphelenchus, Cephalobus,* and *Acrobeles,* and 3 species of fungi, *Fusarium moniliforme* Sheld., *F. vasinfectum* Atk., and *Glomerella gossypii* Edgerton, in a study of the role of nematodes in damping-off of cotton seedlings. Although large numbers of nematodes of the species with which they were dealing may have a stunting effect on the plants, their experiments failed to show that any of these nematodes were factors of primary importance in damping-off of cotton.

Arndt and Steiner (2) also studied the role of several nematodes (other than root-knot) and various damping-off fungi in relation to cotton seedling losses, particularly during the early part of the growing season. *Aphelenchus parietinus* Bastian (now *Aphelenchoides parietinus* (Bastian) Steiner) was largely responsible for the killing of young cotton seedlings during the cold rainy periods that often occur in the early portion of the growing season. Their investigations did not reveal a nematode-fungus disease complex.

Taylor et al (9) noted in their field experiments that carbon bisulfide treatment controlled nematodes sufficiently to permit several Fusarium-wilt-resistant and wilt-tolerant varieties of cotton to become established and make satisfactory growth. In unfumigated plots, only a few plants survived wilt until the middle of July; root-knot nematodes were abundant and many secondary roots were killed by a meadow nematode, *Pratylenchus* sp.

Smith (6) obtained markedly increased cotton

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yields and almost perfect control of Fusarium wilt and nematodes by fumigating the soil with a nematocide. This increase in yield occurred in both wilt-susceptible and wilt-resistant cotton varieties. There were noticeable differences between treatments in rate of growth and amount of wilting early in the season. Both root-knot and meadow nematodes were present.

EXPERIMENTAL RESULTS.—*Controlled inoculum experiment.*—In a screenhouse experiment, virgin desert soil containing a moderate amount of organic matter was steamed for 8 hours at 80°–85° C. In each gallon glazed crock of this soil, 20 acid-delinted Acala 44 cotton seeds were planted in concentric circles with an even spacing to a depth of 1 in. At the time of planting, Rhizoctonia-grain inoculum and cotton root-knot nematodes at the desired rates were added separately to the soil surface of the crocks, and then sufficient water was added to moisten the soil uniformly in all crocks. The treatments in this experiment consisted of no inoculation (controls) and of inoculation with root-knot nematodes alone, with Rhizoctonia alone, and with a nematode and Rhizoctonia combination. Five crocks were used for each treatment. The experiment was conducted during the fall when the maximum and minimum temperatures were similar to those prevailing at the time of spring planting. The average daily range of soil temperatures in the crocks was 21.4°–26.8° C.

The fungus inoculum consisted of a composite of 6 cultures from diseased seedlings. The fungus was grown on autoclaved grain-sorghum medium and then removed from the containers and air dried. Approximately 30 g of grain culture was added to 3000 ml of water and a suspension made in a Waring Blendor. This quantity was added to each crock, since previous pilot tests had shown that it was sufficient to cause the amount of postemergence damping-off desired for this experiment.

Cotton root-knot nematode larvae were hatched by the procedure outlined by Godfrey (3) and modified by Reynolds (5). Approximately 2000 viable larvae in water were added to the soil surface of each crock designated for nematode inoculum.

Five weeks after seeding, the plants were harvested and the roots examined for root-knot nematode and Rhizoctonia infection. Each plant was given a relative root-knot index rating as described by Smith and Taylor (7). The percentages of plants infected by Rhizoctonia were determined.

Although the data summarized in Table 1 were obtained under controlled conditions in only a single experiment, they parallel field observations. An increase in the percentage of plants infected by Rhizoctonia, as compared with the control, was obtained when either nematode or Rhizoctonia inoculum was added, although the mean differences from the control were not statistically significant. Addition of both nematode and Rhizoctonia inoculum resulted in a statistically significant increase in Rhizoctonia disease as compared with the control crocks or those to which only nematodes were added.

TABLE 1.—*Emergence, root-knot indices, and Rhizoctonia infection of Acala 44 cotton*

Treatment	Per centage emergence	Relative root-knot index[a]	Percentage of plants infected with Rhizoctonia
Nematodes alone	78.3	0.81	35.8
Rhizoctonia alone	73.3		48.2
Nematodes and Rhizoctonia	80.3	0.72	64.7
Control (no inoculation)	73.5		31.5
LSD, 0.05	N.S.	N.S.	20.4
LSD, 0.01	N.S.	N.S.	27.3

[a] Relative root-knot index as follows: 0 = no infection, 1 = trace, 2 = light, 3 = moderate, 4 = heavy infection.

The relative nematode infection was only a trace, even though a rather large number of viable larvae had been used. During the course of this experiment, soil temperatures were somewhat below optimum for the cotton root-knot nematode. From more recent studies and observations, it is apparent that had these plants been more heavily infected by nematodes, the incidence of Rhizoctonia disease might have been greater. As shown, some Rhizoctonia infection occurred in the noninoculated plants, probably because soil that had been only steamed was used.

Field surveys.—During the spring of 1953, 2 Acala 44 cotton fields infested with the cotton root-knot nematode were selected for study. Field 1 received a preplanting fumigation treatment with Bromofume (83 per cent 1,2-dibromoethane) at the rate of 3 gal. per acre. The fumigant was applied on a row placement basis, in 2 lines 6 in. from the center of the listed bed. An unfumigated strip 8 rows wide was left as a control near the center of the field. The field was seeded about mid-April. Field 2, in the same locality, was planted at approximately the same time. It was generally heavily infested with root-knot nematodes except for a few areas where a relatively light population was found. No part of Field 2 was fumigated.

Individual plant samples were collected during early June from the fumigated and unfumigated portions of Field 1 and from the lightly and heavily infested areas of Field 2. To obtain random samples, plants located at predetermined intervals in randomly selected rows were collected. Plant samples from each of the 4 areas were bulked and brought into the laboratory, where 2 independent ratings of the incidence of nematode infection and Rhizoctonia disease were given each plant. The relative root-knot index was determined as described for the controlled inoculation experiment. The relative amount of Rhizoctonia damage was determined by scoring the extent of disease on a scale ranging from 0 (all plants healthy) to 8 (all plants dead). In addition, plant heights were determined by measuring the distance from the point

of emergence from the seed to the terminal bud.

The results of the field surveys were analyzed by grouping individual plants as to each separate measurement, relative root-knot and Rhizoctonia indices, and height of plant. Since similar results were obtained from both fields, all plant data were grouped for brevity. The results obtained are presented graphically in Figure 1.

When the plants were grouped as to the root-knot index, it was readily apparent that those with the highest rating were the most severely affected by Rhizoctonia and were shortest (Fig. 1, A). Grouping of plants according to Rhizoctonia index, (Fig. 1, B) showed that the plants that were severely infected by Rhizoctonia had on the average a higher root-knot index and were shorter than those less severely diseased. Heights of individual plants were inversely correlated with the relative root-knot and Rhizoctonia indices (Fig. 1, C).

Fumigation studies in the field.—During the spring of 1954, small-scale field plots were established at the U. S. Field Station, Sacaton, Arizona, to determine the efficacy of a chemical in nematode control and to give additional information on the effects of soil fumigation on the severity of Rhizoctonia disease of cotton. The experiment consisted of 3 treatments with 4 replications in randomized blocks. Each plot was 4 rows wide and 12 ft. long. The land on which this test was established was known to contain a relatively uniform and moderately high cotton root-knot nematode population. The chemical was applied 8 in. deep at staggered 1-ft. intervals, 6 in. from the center of the listed bed. One treatment with Dowfume W-40 was made with a hand injection gun. In the second treatment, holes 1 in. in diameter and 8 in. deep were made at the intervals indicated, and Panacalite[4] saturated with Dowfume W-40 was placed in each and covered with soil. This application rate was equivalent to 10 gal. of Dowfume W-40, the same as in plots receiving liquid Dowfume W-40 only, plus 64 lb. of Panacalite per acre. The third treatment consisted of unfumigated control plots. Acala 44 cotton was planted in the center of the listed bed 17 days after fumigation.

Seedling-emergence counts were made and damping-off losses determined in all plots 21 days after seeding. The relative root-knot indices and yield of lint cotton were determined at the end of the growing season. Rhizoctonia disease was very severe in unfumigated plots but relatively light in plots that received any 1 of the fumigants. Less Rhizoctonia disease occurred in plots in which Dowfume W-40 was included than on the control plots (Table 2, Fig. 2). This would appear to indicate that ethylene dibromide (1,2-dibromoethane) had fungicidal as well as nematocidal properties at the dosage rate used in this experiment, but such has not been found to be the case. Stark and Lear (8), after investigating the fungicidal and nema-

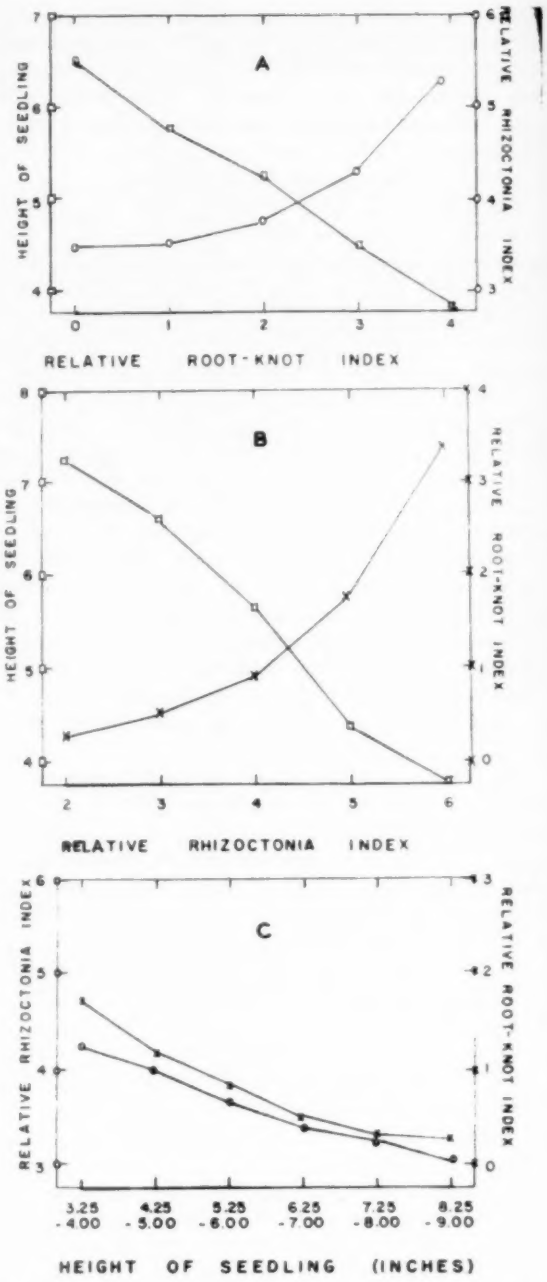

FIG. 1. Relationship among Rhizoctonia disease, root-knot nematode infection, and plant height in cotton seedlings grown in 2 fields, portions of which were fumigated. A) Height (□) and Rhizoctonia indices (o) of seedlings grouped according to root-knot index. B) Heights (□) and root-knot indices of seedlings grouped according to Rhizoctonia index. C) Rhizoctonia indices (o) and root-knot indices (×) of seedlings grouped according to height.

[3] Dowfume W-40 contains 41 per cent 1,2-dibromoethane.

[4] Panacalite is an inert, very absorptive aluminum silicate product used in the building trades. It has no nematocidal or fungicidal action but served only as a carrier for the liquid Dowfume W-40.

TABLE 2.—*Stand loss of Acala 44 cotton from Rhizoctonia disease, relative root-knot index, and yield of lint cotton in fumigated and unfumigated plots*[a]

Fumigant[b]	Loss in stand 21 days after planting	Relative root-knot index[d]	Estimated yield of lint cotton per acre
	Per cent [c]		Lb.
Dowfume W-40	4.8	0.3	1777
Dowfume W-40 + Panacalite	8.1	0.3	1542
None (Control)	47.9	2.4	625
LSD, 0.05	6.7	0.2	416
LSD, 0.01	10.0	0.3	630

[a] Each value is an average of 4 replications.
[b] Both Dowfume W-40 treatments applied at the rate of 10 gal. per acre; the first treatment was injected in the soil by means of a hand gun. In the second treatment, Panacalite at the rate of 64 lb. per acre was saturated with Dowfume W-40 and this material was poured in holes and covered.
[c] Percentage loss is based on total emergence.
[d] Relative root-knot index as follows: 0 = No infection, 1 = trace, 2 = light, 3 = moderate, 4 = heavy infection.

tocidal properties of several chemicals, reported that although ethylene dibromide was the most powerful nematocide included in their studies, it was ineffective in controlling damping-off of pea seedlings. They were not concerned with a nematode-fungus disease complex, but with the effectiveness of the chemicals tested in separate experiments against a seedling disease caused by a species of *Fusarium*.

Fumigated plots yielded considerably more cotton and showed somewhat less root-knot damage than did unfumigated plots (Table 2). The nematode as a debilitating parasite was responsible for part of the difference between the fumigated and the control plots, but most of the difference in yield was brought about by a loss in stand from Rhizoctonia early in the growing season. It appears that the nematode, by the early invasion of cotton seedlings, weakens the plant so that losses due to Rhizoctonia are greatly increased.

Reduction in stand of Acala 44 cotton seedlings in a large-scale fumigation experiment in which Nemagon (1,2-dibromo-3-chloropropane) was included is shown in Figure 3. The greatest loss of plants occurred in unfumigated plots where the root-knot nematode was not controlled. These plants became severely infected with the root-knot nematode and showed a decided reduction in vigor soon after emergence. The seedlings were then attacked by Rhizoctonia and many of them were killed.

Studies of mechanical injury to seedlings.—As pointed out earlier, there are probably numerous factors other than root-knot nematodes (such as temperature, insects, and cultural practices) that reduce seedling vigor and thus make a plant more susceptible to Rhizoctonia. One of these might be mechanical injury to the plant in its early growth stages. This factor was evaluated in an excised-cotyledon experiment

FIG. 2. Rhizoctonia disease on Acala 44 cotton grown in fumigated and unfumigated soil. Three plants on left taken from unfumigated plot show both root-knot nematode and severe Rhizoctonia infection. Note desiccation. Two plants on right taken from fumigated plot show no root-knot nematode and only slight Rhizoctonia infection.

at Sacaton in 1954, in a portion of a block that had been planted to Pima S-1 cotton. The soil had been determined to be free of root-knot nematodes.

Pima S-1 cotton, *G. barbadense* L., was seeded on April 21. After 10 days, the seedlings in small plots, each containing approximately 5 linear row feet, were thinned to 20 seedlings per plot. At the same time, portions of the cotyledons were removed with shears as follows: 1) both cotyledons, 2) 1½ cotyledons, 3) 1 cotyledon, 4) ½ of 1 cotyledon, and 5) no portion of cotyledons (control). Seedlings that emerged after these treatments were pulled and discarded. The experiment consisted of 3 replications in randomized blocks.

On June 17, a survival count was made in each plot. The plants were then harvested and brought to the laboratory for further study. There, additional data were secured, namely, height, stem cross-sectional area, and severity of Rhizoctonia disease. No plants survived when both cotyledons were removed (Table 3). This total loss occurred within 10 days after treatment. The loss was probably due to plant desiccation following treatment and not to attack by Rhizoctonia; such treatment proved to be too severe under low hu-

FIG. 3. Acala 44 cotton growing on fumigated and on unfumigated soil. Soil of 12 rows to the left received 0.5 gal. (8.6 lb.) of technical Nemagon per acre as a preplanting treatment for nematode control. Note only slight reduction from Rhizoctonia. Twelve rows to right were not fumigated and show considerable loss in stand from Rhizoctonia.

midity conditions of Arizona. When 1½ cotyledons were removed, less than ⅓ of the plants survived; both plant height and cross-sectional area of stems were much less and Rhizoctonia disease was more severe than in seedlings with no portion of the cotyledons removed. After removal of 1 cotyledon or only ½ cotyledon, plants were somewhat smaller, fewer survived, and they showed a greater incidence of Rhizoctonia disease than the control seedlings.

TABLE 3.—*Survival, growth, and relative Rhizoctonia indices of mechanically injured Pima S-1 cotton seedlings*[a]

Type of mechanical injury	Plants surviving	Plant height	Cross-sectional area of stem	Relative Rhizoctonia index[b]
	No.	Cm	Mm^2	
Removal of both cotyledons	0			
Removal of 1½ cotyledons	6.3	7.5	4.2	3.7
Removal of 1 cotyledon	10.0	8.2	5.0	3.1
Removal of ½ of 1 cotyledon	13.7	9.8	5.3	2.7
None (Control)	17.3	12.6	6.0	1.7
LSD, 0.05	3.8	1.8	0.6	0.5
LSD, 0.01	5.5	2.5	0.9	0.7

[a] Each value is an average of 3 replications.
[b] Relative Rhizoctonia index ranges from 0 (all plants healthy) to 8 (all plants dead).

A similar experiment was conducted simultaneously at Scott, Mississippi; the treatments were the same as described. Results paralleled very closely those obtained at Sacaton, except that under the more humid conditions of Mississippi a few plants survived when both cotyledons were removed. Results obtained from these experiments indicate further that factors affecting plant vigor are associated with the Rhizoctonia disease of cotton.

U. S. DEPARTMENT OF AGRICULTURE
SACATON, ARIZONA

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EFFECT OF AMINO ACIDS ON THE VIRULENCE OF HELMINTHOSPORIUM SATIVUM TO WHEAT SEEDLINGS [1]

S. B. Hrushovetz [2]

SUMMARY

Progressive attenuation of the virulence of *Helminthosporium sativum* Pam., King & Bakke through 6 successive transfers was effected by adding 0.1 per cent of any one of the amino acids alanine, arginine, histidine, isoleucine, leucine, methionine, serine, threonine, and tryptophane to Czapek's agar medium upon which the pathogen had been cultured before it was used in making soil inoculum.

An hypothesis based on a modification of the nutritional theory of parasitism together with the phenomenon of heterokaryosis is offered to account for this decrease in virulence. It is postulated that the specific amino acid supplement effects a selection from the heterokaryon and results in the predominance of those strains that require this amino acid for growth. If this amino acid is available in the host tissues when the selected strain is applied as inoculum, the fungus may develop, but it is relatively nonpathogenic. If the specific amino acid is absent or unavailable, then the pathogenic component of the heterokaryon is favored.

Among plant pathogenic bacteria, changes in pathogenicity resulting from mutation have been noted, and the mutation has been shown to be sometimes associated with specific nutrients in the medium. Van Lanen et al (15) obtained attenuated strains of the tumor-forming bacterium *Agrobacterium tumefaciens* (E. F. Sm. & Towns.) Conn by repeated transfers to a medium containing mannitol, a nitrate, mineral salt, and 0.3 per cent glycine. The resulting bacterial cultures failed to induce galls on punctured areas of tomato plants. The virulence of the organism was subsequently restored by repeated subculturing in a yeast medium. These authors found that all aliphatic amino acids as well as lysine were attenuators, but other diamino acids and dicarboxylic acids were inactive. Braun et al (1), working with the animal pathogen *Brucella abortus* Schmidt & Weis, induced a change from smooth to rough type of bacterial culture by using alanine in the culture medium. With increased alanine concentration, new types of cells developed that were antigenically different from the rough or smooth types.

Wheat roots have been shown to be rich in aliphatic amino acids (6). These compounds have been detected in water in which the roots were suspended (L. E. Tyner, unpublished data) and in the rhizosphere when the plants were grown in soil (10). It is possible that the amino acids may directly affect the growth and virulence of soil microorganisms that come into contact with the roots. The present paper reports the results of studies on the influence of amino acids on the pathogenicity of *Helminthosporium sativum* Pam., King & Bakke, an important fungus pathogen of cereal roots (2, 3).

Materials and methods.—Czapek's medium of the following composition was used as a basic substrate: $NaNO_3$ — 2 g; K_2HPO_4 — 1 g; $MgSO_4 \cdot 7H_2O$ — 0.5 g; KCl — 0.5 g; $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ — 0.01 g; sucrose — 15 g; Bacto agar — 15 g; distilled water to 1 liter.

Amino acids secured from Nutritional Biochemicals Corporation, Cleveland, were added singly in concentrations of 0.1 per cent (w/v) to the liquified medium,

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and the mixture was dispensed in test tubes in 10-ml quantities. After sterilization, the tubes were slanted, cooled, and seeded with *H. sativum*. Single-spore transfers were not used in any part of the experiment. Subcultures were made after 2 weeks by mass mycelial transfer from these tubes to freshly prepared test-tube slants, and this process was repeated 6 times. An aqueous suspension of the fungal tissue in each tube was made and divided equally among 6 flasks, each containing approximately 125 g of a mixture of 3 parts of black soil, 1 part of sand, and 2 per cent by weight of cornmeal. The flasks containing this mixture were sterilized previously at 15 lb. pressure for 4 hours. Following 2 weeks' incubation, 10 surface-sterilized seeds of Red Bobs wheat were placed in each of 3 of these flasks. The inoculum in the remaining 3 flasks was separately diluted with 8 parts of a nonsterilized 3:1 soil-sand mixture in 6-in. unglazed pots. Before this dilution, a portion of the fungus mycelium present in each flask of soil inoculum was removed, placed in lactophenol on a microscope slide, and examined to determine the presence of spores. Two days after the inoculum was added, 10 surface-sterilized Red Bobs wheat seeds were placed in each pot at a uniform depth of 1½ in.

Data on infection and on plant height were secured after 2 weeks' growth of the seedlings. To estimate infection, a disease rating index was prepared similar to that used by Greaney et al for mature plants in their field experiments (5). A combination of several symptoms, including coronal lesions and cortical rot, as well as plant height, were used in determining the severity of infection, which was indicated by assigning ratings between 0 and 100. For the flask experiment, plant height was not used in the index, since no water was added to them and they were kept in the dark during the 2-week period of growth of the seedlings.

TABLE 1.—*Relative virulence of Helminthosporium sativum to wheat seedlings grown in unsterilized soil after each of 6 serial transfers of the pathogen to Czapek's medium containing various single amino acids*

Amino acid	Infection rating[a] of seedlings grown in soil infested with *H. sativum* after indicated transfer[b]					
	1	2	3	4	5	6
DL-Alanine	41	21	27	13	10	0
L-Arginine	87	48	55	53	5	0
DL-Aspartic acid	79	68	71	92	35	69
L-Glutamic acid	82	77	90	92	46	63
Glycine	91	48	90	92	46	66
L-Histidine · HCl	46	17	41	21	15	14
DL-Isoleucine	95	31	82	53	37	8
DL-Leucine	78	73	41	29	12	5
L-Lysine · HCl	93	63	98	46	46	40
DL-Methionine	68	29	46	22	5	4
DL-Norleucine	84	64	95	99	45	51
DL-Phenylalanine	89	53	94	86	39	49
DL-Serine	76	28	38	31	3	5
DL-Threonine	79	42	38	36	11	1
DL-Tryptophane	96	64	99	42	36	2
L-Tyrosine	99	40	95	100	39	73
DL-Valine	93	43	95	98	54	69
None	96	47	99	93	65	62

[a] Severity of infection rated on a scale from 0 to 100, with 0 indicating no disease and 100 indicating dead plant. Each figure is the average of 3 replicates.
[b] Transfers were made at 2-week intervals.

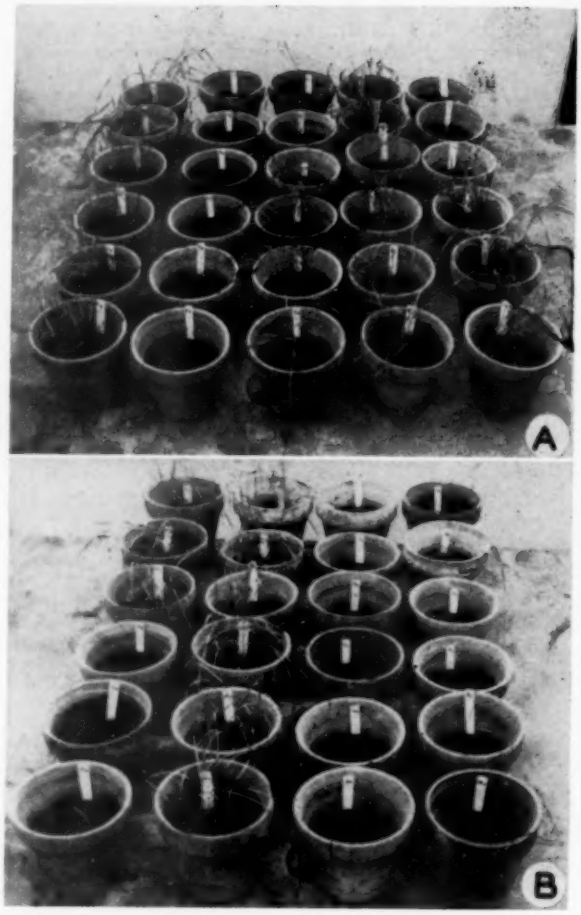

FIG. 1. Wheat seedlings after 2 weeks' growth in soil infested with *Helminthosporium sativum* from cultures grown on Czapek's medium to which the various single amino acids had been added. A) Back 3 rows (left to right): alanine, aspartic acid, glycine, leucine, norleucine. Front 3 rows (left to right): arginine, glutamic acid, histidine, isoleucine, methionine. B) Back 3 rows (left to right): serine, tryptophane, valine, lysine. Front 3 rows (left to right): phenylalanine, threonine, tyrosine, none.

RESULTS.—In the pot experiments, the virulence of the pathogen *H. sativum* was markedly attenuated by the addition to the culture medium of the amino acids alanine, arginine, histidine, isoleucine, leucine, methionine, serine, threonine, or tryptophane, but not by the addition of other amino acids tested (Table 1). In general, there was a progressive decrease in virulence with successive transfers to these media. Growth of the wheat seedlings in the sixth successive planting is shown in Figure 1. No plants appeared above the soil surface in several treatments, but treatments in which attenuation of virulence of *H. sativum* occurred were

characterized by a high percentage of emergence and a vigorous growth of the seedlings.

In the experiment in which wheat seedlings were grown in flasks containing undiluted inoculum (Table 2) the attenuation of virulence of the pathogen was not so pronounced as in the pot experiment, but a similar trend was observed. Again, the change in virulence appeared to be progressive. The attenuation in this case could not have been related to antibiotic effects by other microorganisms (14), since only *H. sativum* was present. Moreover, it is improbable that antibiotic effects were of importance in the pot experiment, where unsterilized soil was used for diluting the inoculum, because the microflora associated with *H. sativum* in the different treatments should be common to all. Microscopic examination of the contents of the flasks of soil inoculum showed that spores were present in all but 4 treatments, namely, those where threonine, lysine, isoleucine, or methionine was added. No relationship was found between the prevalence of either spores or mycelium and the virulence of the culture. Growth of wheat seedlings in flasks of undiluted soil inoculum of *H. sativum* for 2 amino acid treatments is shown in Figure 2.

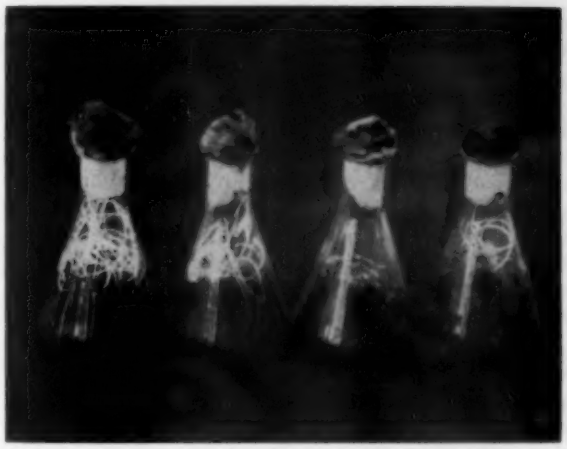

FIG. 2. Wheat seedlings after 2 weeks' growth in undiluted soil inoculum of *H. sativum* prepared from cultures grown on Czapek's medium containing amendments of single amino acids. Left to right: Flasks 1 and 2, methionine amendment; Flasks 3 and 4, glycine amendment. Note marked attenuation of virulence with the methionine supplement.

DISCUSSION.—The methods employed suggest that the amendments affected the physiology of the pathogen rather than that of the host. According to the balanced theory of parasitism as outlined by Lewis (11) ". . . . a parasite grows in a particular host or organ because in that host or organ there is available to the parasite the kinds and quantities of foods that are necessary for its life, and in resistant hosts or organs the necessary kinds and/or quantities are not present. The possible combinations of foods present plus the capacity of the parasite to respond differently to these combinations accounts for the varying degrees of resistance and susceptibility. . . ." The present author proposes a modification of this theory, which together with the phenomenon of heterokaryosis, may account for the variation in pathogenicity of *H. sativum* observed in this study.

According to this hypothesis, the heterokaryons present in *H. sativum* grown on potato-dextrose medium include a mixture of strains of the wild type and those favored by or dependent on specific amino acids for growth. Czapek's medium favors the wild-type strains, whereas the same medium with a specific amino acid supplement selects from a heterokaryon those strains that require this amino acid for growth. The pathogenicity on a susceptible host of such amino-acid-dependent strains will be influenced by the presence and availability of the specific amino acids in the tissues of that host. If the amino acid is present, the predominant mutant type develops at the expense of the wild type when the inoculum contains a mixture of mutant and wild-type nuclei. Conversely, if the amino acid is absent from the host, the wild-type nuclei will develop.

Genetic studies by Jinks (8) and Olive (12) showed that heterokaryons were more stable on media rich in nutrients than on media low in nutrients. Jinks (9) also found that in a heterokaryon of *Penicillium cyclopium* Westling not only was the ratio of the 2 kinds of nuclei of the homokaryons comprising the heterokaryon characteristic for a specific medium, but it was also related to the comparative growth rates of the 2 homokaryons on that medium. Hrushovetz (7)

TABLE 2.—*Infection ratings of wheat seedlings grown in flasks containing a soil-sand-cornmeal mixture infested with Helminthosporium sativum following each of 6 serial transfers of the pathogen to Czapek's medium containing various single amino acids*

Amino acid	Infection rating[a] of seedlings grown in mixture infested with *H. sativum* after indicated transfer[b]					
	1	2	3	4	5	6
DL-Alanine	92	72	44	86	31	48
L-Arginine	100	98	68	97	27	61
DL-Aspartic acid	91	100	96	90	66	99
L-Glutamic acid	100	100	98	100	63	83
Glycine	95	100	97	98	64	99
L-Histidine · HCl	65	90	84	86	27	26
DL-Isoleucine	87	79	93	82	23	22
DL-Leucine	97	100	81	72	31	25
L-Lysine · HCl	98	97	93	100	93	73
DL-Methionine	89	91	66	58	26	4
DL-Norleucine	93	97	98	87	97	17
DL-Phenylalanine	98	100	99	99	99	72
DL-Serine	99	93	69	82	35	61
DL-Threonine	96	98	89	89	57	77
DL-Tryptophane	99	100	63	86	100	83
L-Tyrosine	100	100	97	100	99	93
DL-Valine	100	97	94	99	100	93
None	98	99	93	100	99	98

[a] Severity of infection rated on a scale from 0 to 100, with 0 indicating no disease and 100 indicating dead plant. Each figure is the average of 3 replicates.

[b] Transfers were made at 2-week intervals.

has shown cytologically that heterokaryons may exist in *H. sativum*, and Dent et al (4) found that potatoes are a rich source of amino acids. The original isolate (H-44) used for the present experiment had been cultured on potato-dextrose agar (PDA) for several years. Strains of this fungus favored by or dependent upon amino acids could readily be established by growth on PDA for such a period. Growth of such heterokaryons on media other than PDA would result in a selection of strains dependent on the various substances present in the media. Thus, whereas only wild-type strains would grow on Czapek's medium, both the wild type and biochemical mutant strains requiring a specific amino acid would grow on this medium supplemented with that amino acid. Furthermore, according to the findings of Jinks (9) mentioned above, there should be present a higher percentage of nuclei of the mutant type than of the wild type on the modified Czapek's medium. Katznelson and co-workers (10, 13) and Tyner (unpublished data) have shown that wheat roots may excrete various amino acids, and Hrushovetz (6) has shown chromatographically that at least 11 α-amino acids occur in the free state in wheat roots. According to the nutrition-balance hypothesis of parasitism (11), the degree of colonization of the wheat seedling by the different "amino-acid-requiring" mutant strains as compared with the wild-type strains will depend on the presence and availability of the amino acids in the rhizosphere and in the tissue of the host. If the particular amino acids required by such mutants are absent, or if present are unavailable to the pathogen, only mycelium containing wild-type nuclei will colonize the host. If the ratio of wild-type to such mutant nuclei is high, severe infection will occur. The converse is also true. After each subculturing on Czapek's medium containing an amino acid supplement, the heterokaryon will possess fewer nuclei of the wild type and more of the mutant requiring this amino acid for growth. Consequently, less infection should occur with each subculture. The progressive loss of virulence with successive transfers of cultures (Table 1) tends to substantiate the thesis of such a nutritive selection of the mutant homokaryon. It is for this reason that the author has called the phenomenon "apparent attenuation." The amino acids do not act as mutagens but rather as selective agents on mutants that have occurred spontaneously earlier in the life history or possibly during growth of the fungus on such media. The complete absence of infection in some tests in the sixth transfer would suggest that the heterokaryons had dissociated and only the homokaryotic "amino-acid-dependent" strain was present.

With *Agrobacterium tumefaciens*, attenuation in pathogenicity is reversed by growing the organism on a medium that lacks the amino acid amendment, but it is not known if this reversion occurs in *H. sativum*.

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GRAIN STORAGE STUDIES XXIII. TIME OF INVASION OF WHEAT SEED BY VARIOUS SPECIES OF ASPERGILLUS RESPONSIBLE FOR DETERIORATION OF STORED GRAIN, AND SOURCE OF INOCULUM OF THESE FUNGI[1]

John F. Tuite [2] and Clyde M. Christensen [3]

SUMMARY

Seeds of wheat were collected from ripe plants and from plants left either standing, shocked, or windrowed in the field for as long as a month after ripening. The seeds were treated lightly with sodium hypochlorite solution and cultured on malt-salt agar designed to disclose the presence of storage fungi. The collections comprised different varieties and classes of wheat from several different states, and were made over a period of 3 harvest seasons during which the weather at and following harvest ranged from moderately dry to wet. Only a small percentage of the seeds were infected by storage fungi. Inoculation of the standing ripe grain prior to sampling did not greatly increase the percentage of seeds infected with storage fungi. Seeds in the heads and seeds threshed from the heads were stored together in moist chambers at relative humidities favorable to the development of storage fungi; the threshed seeds were invaded by storage fungi much more rapidly than seeds in the heads. Inoculum of storage fungi was uncommon in the air in ripe wheat fields, moderately abundant in the air in country elevators where freshly harvested grain was being handled, and much more abundant in the air of a terminal elevator. Percentage of wheat seeds yielding storage fungi increased considerably between harvest and arrival of the grain at a terminal.

Invasion of stored seeds by various species of the genus *Aspergillus* is now known to be a primary cause of, or major contributing factor to, the development of germ-damaged or sick wheat in stored grain (1, 3, 4) and of various biochemical changes that accompany such deterioration (1, 6). The species of *Aspergillus* involved in the deterioration of stored malting barley rarely invade the seed except after harvest (9). Presumably the same would apply to wheat, but this is not definitely proved. Fungus mycelium is common under the pericarps of wheats grown throughout the world (7), but most of this mycelium appears to be that of Alternaria and other fungi that do not grow in grain at the moisture contents ordinarily encountered in storage (2, 8). It therefore seemed to be of some importance to determine when wheat seed became infected with storage fungi, and to explore some of the possible sources of inoculum of these fungi.

LEVEL OF INFECTION IN FIELDS.—In the growing season of 1953, heads of wheat were collected by cooperators in grain fields in Kansas, Missouri, Iowa, Illinois, Ohio, and New York at approximately weekly intervals from the late milk stage to ripening. These were sent immediately by air mail to our laboratory in St. Paul, Minnesota. From 50 to 100 seeds were removed from the heads of each sample and either washed in running water to which a detergent had been added or immersed in 1.0 per cent sodium hypochlorite for 1 minute. They were then rinsed in sterile water and cultured on malt agar containing 7.5 per cent sodium chloride. In nearly all cases, less than 5 per cent of the seeds so cultured yielded species of *Aspergillus*, although species of *Alternaria* was recovered from 90–100 per cent of them. One exception was a sample of several heads containing mature seeds that had been wrapped in waxed paper and sent by first class mail so that the heads were for several days in a warm and humid atmosphere. Species of *Aspergillus* known to be involved in the deterioration of stored grain were recovered from 100 per cent of these seeds after they were treated with hypochlorite solution. These preliminary results indicated that up to harvest time very few wheat seeds had been invaded more than superficially by storage fungi. It was thought possible, however, that under some circumstances wheat seed during or after ripening might become more extensively invaded by storage fungi while the seeds still were in the heads.

From 1953 through 1955, 72 samples, each consisting of 2–20 heads, were collected during and after ripening. Most of these were hard red spring and winter varieties, but some of durum also were taken. They were collected chiefly in Minnesota and eastern North Dakota, but some came from Nebraska, Illinois, Ohio, and Missouri. Some came from wheat plants that remained in the field for a month after normal harvest time. The weather during and subsequent to harvest, in the areas where the heads were collected, ranged from dry to moist.

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From 50 to 100 seeds were removed from the heads of each sample, immersed in 1.0 per cent sodium hypochlorite for 1 minute, rinsed in sterile water, and cultured on malt agar containing 7.5 per cent sodium chloride. Approximately 9000 seeds were so cultured. *Aspergillus glaucus* Link was obtained from less than 0.1 per cent, and *A. flavus* Link, *A. candidus* Link, and species of *Penicillium* combined from less than 0.2 per cent of the seeds. The sample highest in storage fungi yielded species of *Aspergillus* (chiefly *A. glaucus*) from only 4.0 per cent of the surface-treated seeds. Wheat variety or class, geographical location, or weather that prevailed during the harvest season appeared to have no detectable influence on the prevalence of infection of the seed by species of *Aspergillus*. Grain that remained windrowed, shocked, or standing in the field for a month beyond the normal harvest season was essentially free of infection by storage fungi. Substitution of 0.5 per cent sodium hypochlorite for ½ minute for the usual 1.0 per cent for 1 minute failed to disclose a larger percentage of seeds invaded by storage fungi.

NATURAL FIELD CONTAMINATION.—It was thought likely that spores of storage fungi might be present in considerable numbers on the outside of the seeds at ripening time. To explore this, heads of ripe wheat were collected and the seeds removed with sterile tweezers and placed without surface treatment on malt agar containing 7.5 or 15 per cent sodium chloride. Agar containing 15 per cent sodium chloride was used because it was known to inhibit the growth of fungi such as Alternaria and to permit the development of various members of the *A. glaucus* group known to be among the most important of the storage fungi (3, 4). Twenty-seven samples of wheat were so tested. These included 7 varieties of hard red spring, 1 of hard red winter, and 2 of durum. Geographically, the collection sites ranged from the experimental plots at St. Paul to farmers' fields in the Red River Valley of northwestern Minnesota and eastern North Dakota. In the Red River Valley in 1955, the harvest season was delayed 1–2 weeks by wet weather.

Of the approximately 1000 seeds so cultured (without surface treatment) on agar media designed specifically to disclose the presence of storage fungi, an average of 4.8 per cent yielded storage fungi. The highest obtained from a single sample was 20 per cent. There was no statistically significant difference in percentage of seeds yielding storage fungi among 6 different hard red spring varieties grown side by side in 1/40-acre plots near the experiment station at St. Paul. The seed collected in St. Paul, where the harvest season was relatively dry, and that collected in the Red River Valley, where the harvest season was wet, did not differ in the percentage that yielded storage fungi.

Seemingly, inoculum of storage fungi, whether mycelium or spores, was rather sparse on wheat seed up to the time of harvest. To approach this in a different way, ripe seeds were removed from wheat heads by the use of sterile forceps, and each seed was placed on sterile cotton in a tube containing a saturated salt solution at the bottom to maintain a relative humidity of 75–85 per cent in the air of the tube. Each tube was closed with a sterile rubber stopper. Nearly 300 seeds were so collected. The tubes were stored in the laboratory, at room temperature, for 5–6 months. Presumably, if inoculum of storage fungi had been present on the seed in even small amounts when the seed was removed from the head, the seed would have been invaded during subsequent storage for 5–6 months at moisture contents and temperatures known to promote invasion of the seed by these fungi, and such invasion could have been detected readily by culturing the seeds. At the end of the storage period, the seeds were immersed in 1.0 per cent sodium hypochlorite for 1 minute, rinsed in sterile water, and cultured on malt agar containing 7.5 per cent sodium chloride. From 1 sample, after storage for 5 months under conditions favorable to the development of storage fungi, nearly 30 per cent of the seeds yielded storage molds; from the other 5 samples, *A. glaucus* was recovered from only about 4 per cent of the seeds (Table 1). Other than Penicillium, which was recovered from a few seeds of 1 sample, no additional storage fungi were present in the seeds.

A considerable range of geographic areas, weather conditions, and varieties were sampled in the various tests described above, and the results warrant the general conclusion that storage fungi normally do not invade wheat seed to any significant degree before harvest.

INOCULATION OF WHEAT IN THE FIELD WITH STORAGE FUNGI.—Wheat plants in field plots were inoculated with suspensions of spores of *A. glaucus*, *A. candidus*, *A. flavus*, and *A. versicolor*. Each ml of inoculum, when diluted and cultured on malt-salt agar, yielded from 60,000 to 130,000 colonies of each species of fungus. The spores were sprayed onto the heads, usually in the evening, at intervals from just before until just after ripening. Essentially the same tests were made in each of 3 successive years. In one of these years, moist weather prevailed at the test site during and after ripening. In some of the tests, heads of the sprayed plants were covered with plastic bags to maintain a high relative humidity. Seeds were collected from a few days to more than a month after ripening, immersed in 1.0 per cent sodium hypochlorite

TABLE 1.—*Percentage of seed yielding storage fungi after seed had been removed from heads aseptically and stored in sterile tubes at relative humidities of 75–93 per cent for 5–6 months*

Wheat variety	Number of seed	Months stored	Relative humidity (per cent)	Percentage of seed yielding	
				Aspergillus glaucus	Penicillium
Minter	76	5	80	28	0
Willet	53	5	80	6	0
Henry	59	6	75	2	0
	42	6	80	8	0
	51	6	93	6	4

TABLE 2.—*Invasion by Aspergillus glaucus of seed in heads or threshed out and stored for different lengths of time at relative humidities favorable for the growth of storage fungi*

Wheat variety	Relative humidity (per cent)	Length of time stored	Percentage of seed yielding *A. glaucus* after storage as indicated: In heads	Threshed out
Willet	80	2 Months	18	60
Minter	75	4 Months	19	79
	94–100	10 Days	0	13
Minhardi	80–98	10 Days	2	48

for 1 minute, and cultured on malt-salt agar. Approximately 1800 seeds from such sprayed plants were cultured. The greatest amount of infection by storage fungi detected was 8.0 per cent in 1 sample; all other samples yielded storage fungi from an average of less than 1.0 per cent of the seeds. Thus, even when inoculum of storage fungi was applied in abundance to the heads of standing wheat and conditions favorable for the development of storage fungi prevailed, relatively little invasion of the seeds resulted. So long as the seeds remained in the heads they appeared to be resistant to or protected from invasion by these fungi.

In another test, spikes of 3 varieties of wheat, containing ripe seeds, were harvested in each of 3 different years, from 1953 through 1955. A portion of each sample was threshed by hand or with a small head thresher. The threshed seeds and the heads containing ripe seeds were stored together for from 10 days to 3 months in desiccators with salt solutions that maintained relative humidities of 75, 80, 93, and 100 per cent. The seeds were then immersed in sodium hypochlorite, rinsed and cultured on malt-salt agar. In all cases, the threshed seed was invaded more extensively and more rapidly than were the seed in heads (Table 2). Even high humidities, such as might prevail with wet weather after the grain is windrowed, did not promote any appreciable amount of invasion by storage fungi of seeds still in the heads. This is supported by data not here reported. We have cultured many samples of both wheat and barley that had lain in windrows during wet weather for as long as 2 weeks after being cut and never have found any significant number of the seeds invaded by storage fungi.

INFECTION SUBSEQUENT TO HARVEST.— In our work with commercial grain storage firms, we occasionally have encountered parcels of wheat, harvested only 2 weeks before, that upon arrival at the terminal had a high percentage of the seeds invaded by storage fungi. Since seed at harvest time appeared to be relatively free of infection by, and even of inoculum of, these fungi, samples of grain taken between the field and the terminals were tested to obtain information on probable sources of inoculum and on time of infection.

TABLE 3.—*Isolation of storage fungi from non-surface-treated wheat seed collected at various places from the field to the terminal*

Sample source	Number of samples	Number of seeds	Percentage of seeds yielding indicated fungi: *Aspergillus glaucus*	Other storage fungi
Heads of standing, shocked, and windrowed wheat	27	2050	4.8	2.7
Combines	7	1000	4.8	6.8[a]
Country elevators, new crop	16	800	50.0	8.0
Trucks from country elevators unloading at terminal	12	575	64.0	6.0

[a] Mostly species of *Penicillium*.

Samples of wheat were collected from combines, from country elevators near where the combines were operating and to which grain then harvested was being hauled, and from trucks bringing wheat of the same regional crop to the terminal. The last-named samples were taken as the trucks were unloading at a terminal in Minneapolis. Thirty-five samples were so taken. From 50 to 100 seeds of each were placed, without surface treatment or washing, on malt agar containing 15 per cent sodium chloride.

The number of non-surface-treated seeds yielding storage fungi increased between the field and the arrival of the wheat at the terminal (Table 3). This suggests that either inoculum, or invasion of the seed by mycelium, or both, may increase between the time wheat is harvested and the time it arrives at the terminal. By the time the lots of grain here studied had arrived at the terminal, sufficient inoculum was present to permit rapid invasion of the seeds if the conditions of subsequent storage favored such invasion.

SOURCE OF INOCULUM.—To determine where and in what numbers the spores of some of the common storage fungi might be present in the air, the air was sampled either by exposing for 12 minutes petri dishes containing malt agar with either 7.5 or 15 per cent sodium chloride or by taking ½ cu. ft. of air with a volumetric sampling device and culturing dilutions of the spore suspensions so obtained. Plates were exposed (or volumetric samples taken) in fields of ripe wheat, near the grain spouts of combines that were harvesting wheat, in country elevators to which this wheat was being hauled, and in a number of different places in a terminal elevator. Evidently inoculum of storage fungi is relatively uncommon in fields of ripe grain, is considerably more abundant in country elevators, and even more abundant in terminal elevators (Table 4). Incidentally, the terminal elevator sampled in this work was one of the cleanest we have visited.

From this it seems likely that 1 major source of inoculum of storage fungi is material in the elevator it-

TABLE 4.—*Numbers of colonies of storage fungi obtained from approximately ½ cu. ft. of air in grain fields, in country elevators, and in a terminal elevator*

Sampling site	Number of samplings	Average number of colonies per ½ cu. ft. of air in grain fields			
		Aspergillus glaucus	*Aspergillus candidus*	*Aspergillus flavus*	*Penicillium* spp.
Ripening or ripe wheat	11 Fields	0.5	trace	trace	0.5
Exhaust chute of combine	4 Combines in different fields	1.3	1.3	0	6.6
Near moving grain in country elevator, harvest year moist	7 Elevators	524	173	12.3	7.4
Near moving grain in country elevator, harvest year dry	3 Elevators	553	0	249	570
Terminal elevator					
Blending hopper	1	25,000	25,000	10,800	293,100
Above moving, empty conveyor belt	1	1,392	240	48	7,410
Above moving conveyor belt carrying wheat	2	5,900	402	24	8,500
Above moving conveyor belt carrying barley	1	1,344	84	60	24
Near grain-weighing site	1	17,280	3,600	1,400	41,760
Near wheat discharge chute	1	1,220	108	0	1,640
Near grain car being loaded	1	10,960	720	0	48,960

self. To one familiar with the conditions that prevail in many commercial elevators where grain is stored in bulk, this is not surprising. Where large bulks of grain are stored with an average moisture content just below (and sometimes just above) that which permits invasion of the seed by storage fungi, and where conditions often are such that some translocation of moisture from one portion of the grain to another occurs, some growth and sporulation of storage fungi is almost inevitable. It is not unusual to find storage fungi developing rather extensively in the grain in some portions of a grain car when it arrives at a terminal elevator from a country elevator. We have repeatedly observed rather heavy growth and sporulation of storage fungi in local, restricted portions of bulk stored grain. In some elevators, dust, grain, and refuse are allowed to accumulate in places difficult of access. We have at times found storage fungi growing vigorously in such accumulated refuse. Corn in terminal storage may have a moisture content as high as 18 per cent for some time before it can be dried. Moist grain, of whose keeping qualities the elevator operator is justifiably suspicious, frequently is moved from one bin to another. If the grain is moldy, such transfer contributes a heavy load of inoculum to the air. In tests not reported here, we have found grain dust from terminal elevators to contain extremely large numbers of storage fungi. Since wheat when harvested is relatively free of storage fungi, this abundance of inoculum within places where grain commonly is stored for some time in bulk is obvious evidence that some grain or grain refuse within such storage places is moist enough to permit these fungi to grow and sporulate. Data from previous studies (5) prove that wheat in bulk storage often has, in some portions of the bulk, a considerably higher moisture content than is shown on the elevator operator's records. Conditions that permit development of storage molds occur in both country and terminal elevators, and the major inoculum of storage fungi probably comes from the bulk-stored grain itself.

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BADGER BALLHEAD, A NEW CABBAGE RESISTANT TO YELLOWS AND MOSAIC[1]

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SUMMARY

Badger Ballhead is a new cabbage variety derived from a cross between Wisconsin Ballhead and Wisconsin Hollander. It is homozygous for monogenic resistance to yellows, incited by *Fusarium oxysporum* f. *conglutinans* (Wr.) Snyd. & Hans. This is the highest type of resistance to this disease, and the variety may be expected to show no symptoms of the disease on infested soil under environmental conditions optimal for yellows. The variety is also moderately resistant to mosaic, incited by the combined activity of turnip virus 1 and cauliflower virus 1. Although the level of resistance to mosaic, which is multigenic, is not as high as that to yellows, rigid field tests show it to equal or slightly better that expressed by the yellows-resistant varieties Improved Wisconsin Ballhead and Wisconsin Hollander, and distinctly better than that of the standard yellows-susceptible varieties Ferry Hollander, Oakview Ballhead, and Harris Ballhead. Badger Ballhead conforms rather closely in most of its salient horticultural characteristics with other varieties in the Danish Ballhead group. It is distinct from yellows-resistant varieties in that group, however, in producing smaller heads at maturity. It requires approximately the same number of days to maturity as Improved Wisconsin Ballhead and is some 2 weeks earlier than Wisconsin Hollander.

Wisconsin Hollander, the first cabbage variety resistant to yellows (incited by *Fusarium oxysporum* f. *conglutinans* (Wr.) Snyd. & Hans.) that was released by the Wisconsin Experiment Station, was selected from Danish Ballhead and introduced in 1916. It is some 2 weeks later in maturity than the parent variety and deviates from it also in head shape and size.[2] Resistance to yellows in this variety is polygenic.[3] A new line was later selected from the same parent variety; this approached the parent more closely in horticultural type, and its resistance was monogenic. This second variety was introduced jointly by the U. S. Department of Agriculture and the Wisconsin Experiment Station in 1935 as Wisconsin Ballhead.[4] The type was brought closer to the parent type by further selection from a cross between Wisconsin Ballhead and Wisconsin Hollander. This selection was introduced by the same 2 agencies in 1946 as Improved Wisconsin Ballhead,[2] which is also homozygous for monogenic resistance. The salient characteristics of the 3 resistant varieties and of the parent variety were reported by Walker et al.[2] Whereas Improved Wisconsin Ballhead closely resembled Danish Ballhead in days to maturity and in other important characters, it tended to produce larger heads under comparable growing conditions. In view of the market requirement in certain areas for smaller heads at maturity, selection has been continued in the resistant Ballhead progenies for lines that conform more closely to Danish Ballhead in head size at maturity. In the course of this series of selections, progenies were screened also for mosaic resistance. The final selection was increased in 1951 and submitted for commercial trials in several northern cabbage-growing areas in 1952 and 1953. It was released to seed growers for increase in 1954 and finally released to the seed trade under the name Badger Ballhead in the autumn of 1955.

DESCRIPTION OF BADGER BALLHEAD.—Badger Ballhead (Fig. 1) conforms to most of the salient characteristics of Danish Ballhead previously described.[2] Certain physical measurements were made at Madison, Wisconsin, in 1951 and 1953, in comparison with those of Improved Wisconsin Ballhead and 2 standard varieties within the Danish Ballhead group, Ferry Hollander and Oakview Ballhead (Table 1).

The head weight of Badger Ballhead was in line with that of Ferry Hollander and Oakview Ballhead, but significantly less in each season than that of Improved Wisconsin Ballhead. The stem length was always significantly less than that of each nonresistant variety. With respect to the ratio of polar to equatorial diameter of head, Badger Ballhead resembled Improved Wisconsin Ballhead and Oakview Ballhead, the polar diameter being slightly less than the equatorial diameter. The reverse was true in Ferry Hollander, the head being slightly oblong. Wilth respect to the ratio of polar diameter to core length, Badger Ballhead was similar to Oakview Ballhead and Improved Wisconsin Ballhead whereas Ferry Hollander had a relatively long core. With respect to the ratio of head weight to leaf weight, Badger Ballhead was found to resemble Improved Wisconsin Ballhead, which was shown earlier to have a higher ratio than Danish Ballhead.[2] Badger Ballhead requires approximately the same number of days for maturity as does Improved Wisconsin Ballhead, and is thus some 2 weeks earlier than Wisconsin Hollander.

DISEASE RESISTANCE.—The parents of the original cross Wisconsin Ballhead and Wisconsin Hollander,

[1] Accepted for publication November 7, 1956.

[2] Walker, J. C., R. H. Larson, R. E. Foster, and J. E. Kuntz. 1948. Yellows-resistant cabbage varieties in the Danish Ballhead-Hollander group. U. S. Dept. Agr. Circ. 776, 12 p.

[3] Anderson, M. E. 1933. Fusarium resistance in Wisconsin Hollander cabbage. Jour. Agr. Res. 47: 639-661.

[4] Walker, J. C., and L. M. Blank. 1934. Fusarium-resistant Danish Ballhead cabbage. Jour. Agr. Res. 49: 983-989.

TABLE 1.—*Physical measurements of Badger Ballhead in comparison with those of certain varieties in the Danish Ballhead group, Madison, Wisconsin, 1951 and 1953*[a]

Variety and resistance to yellows	Year of trial	Weight of outer leaves (g)	Weight of head (g)	Diameter of head (cm) Equatorial	Diameter of head (cm) Polar	Stem length (cm)	Core length (cm)	Ratio of polar to equatorial diameter of head	Ratio of polar diameter of head to core length	Ratio of head weight to leaf weight
Ferry Hollander (nonresistant)	1951	1484	3370	20.5	21.4	12.4	15.1	1.05	1.40	2.34
	1953	1395	2318	18.0	19.3	13.7	13.4	1.07	1.44	1.67
Oakview Ballhead (nonresistant)	1951	1654	2993	19.8	19.3	12.2	12.3	0.97	1.60	1.90
	1953	1377	2666	19.0	18.8	11.6	11.7	0.99	1.60	2.25
Improved Wisconsin Ballhead (resistant)	1951	1202	4934	23.1	21.2	8.0	11.8	0.92	1.80	4.10
	1953	1383	3427	21.4	19.5	10.6	12.1	0.91	1.61	2.48
Badger Ballhead (resistant)	1951	1029	3365	21.2	20.5	9.3	12.8	0.97	1.60	3.39
	1953	972	2990	20.5	19.0	9.5	11.9	0.93	1.59	3.08
LSD (19:1)	1951	366	680	1.6	1.6	2.0	1.4	0.07	0.14	0.57
	1953	NS	300	0.6	NS	1.9	0.3	0.02	0.05	NS
LSD (99:1)	1951	483	899	2.2	2.1	2.6	1.9	0.09	0.18	0.75
	1953	NS	454	0.9	NS	2.9	0.4	0.03	0.08	NS

[a] The values are averages from 10 plants in each of 3 randomized replicates.

Fig. 1.—A) Plant type and B) head type of Badger Ballhead.

were homozygous resistant and homozygous susceptible, respectively, for the gene pair controlling monogenic resistance to yellows, although the Wisconsin Hollander variety carries a high level of multigenic resistance to the disease.[3, 4] Since monogenic resistant plants show no symptoms of yellows in the field whereas multigenic resistant plants may do so under high midsummer temperatures, it was desired to have the new variety homozygous for monogenic resistance. The method of assay for resistance was that described and illustrated by Walker.[5] Young seedlings were grown in infested soil in metal trays inserted in soil-temperature tanks controlled at a constant tempera-

[5] Walker, J. C. 1950. Plant pathology. McGraw-Hill, New York. 699 p. (See p. 679).

TABLE 2.—*Mosaic index of Badger Ballhead in comparison with other varieties in the Danish Ballhead group, Madison, Wisconsin, 1953*

Variety	Resistance or susceptibility to yellows	Mosaic index[a]
Harris Ballhead	Susceptible	71
Ferry Hollander	Susceptible	68
Oakview Ballhead	Susceptible	68
Superior Danish	Susceptible	55
Improved Wisconsin Ballhead	Monogenic resistant	53
Wisconsin Hollander	Multigenic resistant	53
Badger Ballhead	Monogenic resistant	46

[a] Plants were placed in 1 of 5 disease classes, 0, 1, 2, 3, or 4, depending on whether symptoms were absent, slight, moderate, severe, or very severe, respectively. The number of plants in each class was multiplied by the class number, the sum of the products was multiplied by 100, and the resulting figure was divided by 4 times the total number of plants.

ture of 24° C. Under these conditions only plants homozygous or heterozygous for monogenic resistance survive. This type of assay is much more reliable than a field test. The F_3 progenies from the original cross were submitted to this test, and only those plants that were homozygous for monogenic resistance were retained for further type selection. At least 50 seedlings were used in each test. The final stock release of Badger Ballhead was tested by this method. Of 110 plants tested, none showed yellows; in contrast, of 63 plants of Golden Acre, a susceptible variety used as a control, all succumbed to yellows.

During the course of the breeding program, progenies were also tested for resistance to mosaic, which is incited by the combined activity of turnip virus 1 and cauliflower virus 1.[6] Seedlings were grown in greenhouse flats, where they were inoculated in the second-true-leaf stage by dusting the leaves with Carborundum and rubbing them with a mixture of extracts of cabbage plants previously inoculated with the 2 respective viruses. Following inoculation, the plants were kept in a cold frame for about 3 weeks then transplanted to the field in mid-June. Fifty plants or more were used in each lot tested, but replicates were not used. In early September, plants were rated for symptom severity and placed in 1 of 5 disease classes, 0, 1, 2, 3, or 4, depending upon whether symptoms were absent, slight, moderate, severe, or very severe, respectively. To secure the index of each lot, the number of plants in each class was multiplied by the class number, the sum of the products was multiplied by 100, and the resulting figure was divided by 4 times the total number of plants. Thus, if all plants were in class 0, 1, 2, 3, or 4, the index value would be 0, 25, 50, 75, or 100, respectively.

As already shown by Pound and Walker,[6] it is possible to raise the level of mosaic resistance, which is a multigenic character, by continuous selection within progenies showing relatively low mosaic indices. The results of the mosaic test of the final stock release of Badger Ballhead in comparison with 4 standard yellows-susceptible and 2 standard yellows-resistant varieties in the Danish Ballhead group are shown in Table 2. Badger Ballhead had the lowest index in the group. Since the mosaic test used results in disease expression that is much more severe than that ordinarily encountered in the field, it is believed that the level of mosaic resistance in Badger Ballhead will be of considerable commercial value.

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OCCURRENCE OF MIXTURES OF TRISTEZA VIRUS STRAINS IN CITRUS [1]

Theodore J. Grant and Richard P. Higgins

SUMMARY

Tristeza virus from selected sources caused very mild, mild, or severe symptoms of stunting, vein and veinlet clearing in the leaves, and pitting of the stems beneath the bark of Key lime test plants. Branch growth of the inoculated Key lime plants was proportional to the severity of symptoms induced by the tristeza virus employed. Repeated selections and transmissions by leaf pieces from mildly affected sources have indicated that mild tristeza virus in Florida is a virus that mutates readily or that exists as a mixture of strains, some of which consistently cause stem pitting and others few or no stem pits on Key lime plants. Cross-protection tests with selected virus sources resulted in varying degrees of protective effects. The explanation for the presence of virus strain mixtures within a given plant appears to be related to the failure of any 1 strain to become thoroughly systemic, thus affording susceptible sites for the development of other strains.

The existence of strains of tristeza virus, some of which cause mild symptoms and others severe symptoms, was demonstrated in Brazil (8). The maintenance there for a 3-year period of sweet orange trees with either mild or severe symptoms on non-tolerant rootstocks and on key lime seedlings, together with the lack of symptom expression with either type of virus strain in sweet orange on a tolerant rootstock, constituted further evidence of the existence of tristeza virus strains (5). Both strains of tristeza virus were obtained through transmissions by means of the aphid vector, *Aphis citricidus* (Kirk.). The freedom of the test plants from the viruses causing psorosis, xyloporosis, and exocortis was indicated by the fact that symptoms incited by these other viruses were not observed in the rootstock tests (9) on plants inoculated by means of aphids. Field observations and tests in Africa (12, 13) and more recently in Texas (17) also present evidence of the existence of tristeza virus strains.

Since the first report from Florida (11) of a strain of tristeza virus that induces relatively mild symptoms on Key lime test plants, its widespread distribution has been demonstrated (2). The present studies demonstrate the existence of mixtures of tristeza virus strains.

METHODS.—Aphid transmission of tristeza virus from scattered grove trees to Key lime plants (15) provided sources of tristeza virus believed to be free of viruses that are not aphid transmitted.

A Key lime plant that became infected after it had been placed close to an aphid-infested Temple orange tree known to be carrying the tristeza virus is referred to as the T_0 source of very mild tristeza virus. Repeated tissue transmissions to Key lime plants from this source consistently resulted in very limited vein-clearing symptoms and very few scattered pits on the stems beneath the bark.

Two Key lime plants infected with tristeza virus as a result of controlled aphid-transmission tests (16) in which the Temple orange field tree mentioned previously was used as source of inoculum were selected as sources of mild tristeza virus, because of their scattered vein-clearing and stem-pitting symptoms. They are herein referred to as T_1 and T_2 sources of mild tristeza virus.

The T_3 source was obtained by tissue and not by aphid transmission from a lime tree in the field, thus it could have been carrying other viruses. The inoculated Key lime test plants, however, showed distinct vein and veinlet clearing with a tendency toward flecking, yellowing, and cupping of the leaves; stunting of growth; and the production of many pits or striations in the stem beneath the bark of the infected plants. These symptoms are typical of a severe tristeza virus reaction on Key lime, but they are not considered to be as severe as those caused by the common or severe tristeza virus strain studied in Brazil.

All tristeza virus sources herein referred to are being tested for the presence of other known citrus viruses. This work has not been completed, but each source is labeled so that it may be referred to in later publication if mixtures with other citrus viruses are involved.

In the comparative tests reported, efforts were made to use comparable Key lime seedling test plants. In general, the inoculations were made with leaf pieces (19), and the top edge of the leaf was left outside the bark flap so that its continued living condition would indicate successful union and potential virus transmission. At the time of inoculation, plants in any given test were cut back to a single main stem and to approximately the same height. Growth measurements of length in cm included only the new branch growth from these main stems. The counts of stem pits were made on comparable representative branches from the upper, middle, or lower portion of the inoculated plants or on all the branches on each plant. Because of differences in plant growth and length of branches, it was necessary to obtain the average number of stem pits for a standard unit. The term stem pit refers to this characteristic symptom of tristeza in Key lime stems from which the bark has been removed.

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RESULTS.—*Comparison of stem growth of inoculated Key lime plants.*—Measurements of the average number and total growth of branches on the noninoculated healthy control and inoculated Key lime plants 4, 6, and 9 months from the date of inoculation from the different virus sources are shown in Table 1. The greatest difference between the inoculated and noninoculated plants occurred in the average total stem growth. It is evident that the effect on growth was more or less proportional to the severity of leaf and stem-pitting symptoms described for the sources employed.

Stem pitting on inoculated test plants.—Observations on the occurrence and abundance of stem pits on Key lime test plants indicated that there were a number of interrelated factors affecting the expression of this symptom. When tristeza-affected field trees were used as sources of virus, some produced more stem pits on the test plants than did others. Repeated collections from a single source tree could also result in some differences in the number of pits on the inoculated plants. A study of the number of pits on each branch of the test plants showed that variations occurred. Plants inoculated with the mild tristeza virus might show an initial shock effect, which was followed by milder symptoms in subsequent growth. The branch first infected was frequently suppressed in growth. Such a branch sometimes showed appreciable pitting, whereas new branch growth had very few pits. There sometimes were cycles of leaf symptoms and stem pitting. In general, growth made in the greenhouse during hot summer weather showed few vein-cleared leaves and less stem pitting than did growth made under conditions of cooler weather and shorter day length of the winter and early spring. For example, comparable branches from Key lime plants inoculated by means of leaf tissue from the mildly affected sources T_1 and T_2 produced an average of 0.5 and 0.4 pit, respectively, per 10 cm of stem in October, whereas comparable readings from the same plants in December averaged 8.5 and 2.7, respectively. In December, the Key lime plants inoculated with the T_3 severe tristeza virus showed an average of 67 pits per 10 cm of stem, and those inoculated with the T_0 very mild tristeza virus had only 0.08 pit. Although variations in the dates of inoculation and size of the plants might account for some variations in stem pitting, the virulence of the virus strain, as indicated by leaf symptoms and growth of the source plants, appeared to be the most important factor controlling the comparative intensity of stem pitting. Study of the data also suggested that variations in the amount of stem pitting on plants inoculated by tissues from the source plants T_1 and T_2 might be due to mixtures of tristeza virus strains in these plants even though they had been inoculated by aphid populations that had acquired the virus from the same field tree.

TABLE 1.—*Branching and stem growth of Key lime plants following leaf-piece inoculation with tristeza virus from different sources*

Source of inoculum	Interval (months)[a]	Average no.[b] branches		Total length branch growth	
		Main stem	Rebranches	Average[b] (cm)	Percentage of check
None (Check)	4	7.9	1.5	104	
	6	9.0	4.6	240	
	9	8.6	11.3	470	100
T_0 (Very mild)	4	6.2	1.4	93	
	6	6.2	15.8	239	
	9	5.8	25.8	459	98
T_1 (Mild)	4	7.6	2.2	62	
	6	7.8	9.8	156	
	9	7.6	13.8	331	70
T_3 (Severe)	4	7.4	2.8	36	
	6	8.6	9.2	62	
	9	7.6	14.2	123	26

[a] Interval between inoculation and taking of data.
[b] Figures are averages for 5 potted plants in greenhouse.

A test was undertaken to determine whether a strain producing many stem pits and one producing no pits could be obtained from these sources. Leaf tissue transfers had been made from the T_1 and T_2 sources to Kalpi lime (Webber's Philippine hybrid) plants and notable differences observed; some plants showed only vein clearing, whereas others had an initial shock reaction and the young terminal growth was yellow.

Progressive selections and leaf-tissue transfers from Kalpi lime plants with marked leaf and stem-pitting symptoms resulted in the obtaining of a strain or strains that caused almost 8 times as many pits in Kalpi lime test plants as did the original inoculum (Table 2). In contrast, selections and transfers from Kalpi lime plants with few to no symptoms resulted in the obtaining of a strain or strains that produced almost no pits on Kalpi lime test plants (Table 2). At the same time, leaf tissue from these same selections from source plants with few pits, when transferred to Key lime plants, resulted in appreciably more pits than did the original. These results may be related to the method of selecting leaf pieces used as inoculum. Leaf-piece selections for increasing stem pits were relatively specific in that they were from the plants and the portion of the leaf showing the most distinct symptoms. In contrast, selections for reduction of pitting were from plants having very few symptoms and by random selection of a leaf from any one of a large number that showed no symptoms. Whether the virus or virus strains were equally distributed in all portions of these symptomless leaf pieces was unknown.

It can be noted (Table 2) that the second selection for increasing stem pits was a plant that had about 9 to 40 times more pits than did the other 3 plants in this series. Observations of the leaves of this second selection source plant indicated that it might be considered slightly off type. In fact, it was at first thought that the great increase in number of pits might be related to a specific sensitivity and symptom expres-

TABLE 2.—*Stem pitting by strains of tristeza virus that were obtained from 2 source plants, by progressive selections and transfers made in an attempt to obtain strains differing in ability to cause stem pitting*

Source[a]	Test plant[b]	No. of stem pits per 10 cm of stem[c] when inoculum was from indicated source[d, e] of indicated test plant				
		In Kalpi lime			In Key lime	
		Original source	First selection	Second selection	First selection	Second selection
		(Following selection for stem-pitting strain)				
T_1	1	2	3	70	4	21
	2	4	4	75	9	27
	3	14	14	133	11	31
	4	26	123[e]	182	28	64
	5	29[d]				71
	Average	15	36	115	13	43
		(Following selection for non-stem-pitting strain)				
T_2	1	0.4[d]	0.0	0.0	0.0	0.7
	2	0.4	0.2[e]	0.0	0.5	2.7
	3	0.5	1.7	0.0	2.5	3.7
	4	1.3	3.2	0.0	4.9	8.9
	5	4.0		0.2		11.8
	Average	1.3	1.3	0.04	2.0	5.6

[a] T_1 and T_2 Key lime source plants were infected with the mild tristeza virus as a result of aphid transmission of the virus from a Temple orange field tree.

[b] Plant numbers in order of increase in average number of pits present.

[c] Three to 4 comparable branches were taken from each plant, the bark removed and the total number of pits and total length of peeled stem recorded, and the average number of pits per 10 cm of stem calculated. The data presented were taken on the same date and were at the following intervals after inoculation: original, 515 days; first selection, 459 days; second selection, 130 days.

[d] First selection based on leaf symptoms 56 days after inoculation.

[e] Second selection based on stem pitting symptoms 329 days after inoculation.

sion on this particular plant rather than any relation to a change in the virus strain. Leaf tissue transmissions from this plant to test plants, however, maintained the same high level of pitting in Kalpi limes and increased the number of pits on Key limes to approximately 3 times more than did inoculations from the first selection (Table 2). These results suggest that dominance of the strain or strains resulting in stem pitting may be influenced by physiological differences in the inoculated plants, but once established, the high level of stem pitting can be reproduced in test plants by leaf-tissue transfers.

The results also suggested the need to determine whether strains might be sorted out from a single source plant. Leaf pieces from the T_0 very mild source were used to inoculate 25 Key lime plants. From this group, the plant showing the most pronounced leaf symptoms and the one showing the mildest leaf symptoms were selected as sources of inoculum for further transmission tests. In like manner, 25 Key lime plants were inoculated from the T_1 source, and from this group the plant showing the mildest symptoms was selected. A strong pitting strain from the T_1 source had already been selected, and the Key lime plant with 71 pits per 10 cm of stem was used. Leaf pieces from each of these sources were used to inoculate series of 5 Key lime plants. The range in the average number of pits per 10 cm of stem on the inoculated Key lime plants in each series follows. When the source was T_0, the first selections for reduction of pits gave 0.0–0.8 pit, and the first selections for increase gave 0.3–4.7 pits. When the source was T_1, first selections for reduction of pits resulted in 3–9 pits, and the third selection for increase produced 61–174. Counting of large numbers of pits was difficult because of their merging into striations. The values presented represent the best estimate. Growth of individual plants in a series was variable, but the data obtained showed that branch growth of the Key limes inoculated from the first and second selections from the T_0 source averaged 85 and 95 per cent, respectively, and growth of plants inoculated from the first and third selections from source T_1 averaged 71 and 51 per cent, respectively, of that attained by the noninoculated controls.

The data from these tests indicate that the mild tristeza virus, as represented by the T_1 and T_2 sources, is a virus that mutates readily or that exists in nature as a mixture or complex of strains, some of which consistently produce numerous stem pits and others few to no pits. The data also indicate that these strains may exist at varying levels in infected plants.

Preliminary tests of cross protection by virus from different sources.—Simultaneous inoculations into each of 20 Key lime plants were made with a leaf piece from the T_3 source and a leaf piece from either the T_1 or T_2 source or from the T_0 source of tristeza virus. For a 6-month period, the doubly inoculated plants showed symptoms characteristic of infection by the T_3 virus.

In another test, Key limes, in series of 5 plants each, were inoculated with leaf pieces from a selected T_1 source showing an average of 11 pits per 10 cm of stem, from a selected T_2 source with an average of 2 pits per 10 cm of stem, and from the T_0 source plant. Four months later, the first 3 Key lime plants in each group were reinoculated with leaf pieces from the T_3 source. At the end of 6 months, it was evident that the inoculations from selected T_1 source (11 pits) had resulted in very little or no protection, those from the selected T_2 source (2 pits) had resulted in some protection, and those from the T_0 source had resulted in considerable protective action. In the last case, the plants were not stunted and had relatively few leaf and stem pit symptoms.

In a third test, 3 series of 10 Key lime plants were inoculated with single leaf pieces from T_0, T_1, or T_2 sources. Seven weeks later, all plants showed some leaves with scattered vein clearing indicative of infection. Four months after the first inoculation, a leaf piece from the T_3 source of tristeza virus was inserted into each of 5 plants of each series. Records taken 8 weeks later showed that the plants inoculated only from the T_0, T_1, and T_2 sources had no to very few symptoms, whereas all plants in the series inoculated also from the T_3 source had 1 to several branches with terminal leaves showing either many or few vein and veinlet symptoms typical of those induced by the virus from the T_3 source. In some cases, these symptoms appeared in the leaves of top branches and in others only in leaves of lower branches. The scattered distribution of branches with leaf symptoms and variations in the intensity of the leaf symptoms suggested an erratic and unequal distribution of the virus from the T_3 source. Whether the strong or the mild symptoms will eventually dominate remains to be determined.

Discussion.—Initial evidence obtained in Brazil indicated that plants invaded by mild tristeza virus were protected against the form that induces severe symptoms (8). More recent tests carried out in Texas showed that for a 14-month period a mild tristeza virus from a Meyer lemon tree inhibited the development of severe symptoms in lime plants bud-inoculated with virus from Sueoka satsuma (17). Information in the present paper shows that tristeza virus strains may exist as mixtures in a plant and suggests that dominance of a mixture of mild strains over a mixture of severe strains may be related to such things as the time of inoculation, the stem-pitting potential or level in the former mixture, and the thoroughness of distribution of the former mixture within the plant. Recently, Costa (3) reported that plants simultaneously inoculated with the mild and severe tristeza virus strains in Brazil produced growth that represented a blending of the individual effects of both strains. In the present studies, it was found that simultaneous inoculation of Key lime plants with leaf pieces from sources with mild and severe tristeza resulted in initial symptoms for 6 months characteristic of those induced by the severe tristeza virus. When Key lime plants were inoculated first with leaf pieces from sources with mild tristeza but with different amounts of stem pitting and then inoculated 4 months later with a severe tristeza virus, the highest degree of protection was secured with the source having the lowest level of stem pitting.

Recently, Sinclair and Walker (18) in field experiments showed that cross protection between strains of cucumber mosaic virus was not complete, presumably because of a failure of any 1 strain to become thoroughly established in all tissues of the cucumber. In greenhouse experiments, they showed that at least 2 strains of cucumber mosaic virus could multiply simultaneously in the same cucumber plant and that complete cross protection between strains did not occur.

The lack of complete distribution of any 1 tristeza virus strain in the Key lime test plants would appear to offer a possible explanation for the occurrence of strain mixtures and the variation in protective effects observed. The tristeza virus is considered to be a phloem virus (4). That the virus is not always equally distributed in all parts of all citrus plants has been shown by the results of tests in Brazil with transmissions from inoculated sour orange and grapefruit plants (4, 6) and in transmission tests in California (20), especially with lemon and grapefruit. Also in the course of investigations of tristeza in Florida, inoculated seedlings occasionally failed to produce symptoms even though the leaf piece used for inoculation remained alive.

Bennett (1), in a discussion of interference phenomena between plant viruses, pointed out that inoculation by means of grafts or vectors, followed by movement of the virus in the phloem, would afford opportunity for the challenging virus to come into contact with sites in which it could be established and multiply. On the basis of observed initial reactions of Key lime plants to inoculations with the challenging severe tristeza virus, it would seem that virus from the T_0, T_1, or T_2 sources had not completely invaded all parts of the Key lime test plants in a 4-month period, thus allowing sites for the development of the challenging virus.

Observed reactions of many citrus plant varieties and hybrids following inoculation with the tristeza virus in Brazil (4, 10) indicated a wide range in the tolerance of different plant tissues. Variations in the time required for symptom expression following inoculation suggested that the rate and amount of virus multiplication varied. In Australia (7) and South Africa (14), it has been noted that virus from sweet orange and mandarin field trees caused more severe symptoms on test plants than did virus from grapefruit, Eureka lemon, or sour orange field trees. These results indicated a qualitative difference in the tristeza virus from different hosts. Fraser (7) attributed the yellowing of test plants to a new virus and named it seedling yellows. Costa et al (6) considered the yellowing one of the symptoms caused by tristeza virus.

McClean and van der Plank (14) also presented evidence that seedling yellows is a symptom of infection by the tristeza virus complex. They suggested that the tristeza virus complex has 2 components: a stem-pitting component, which alone can cause severe stem pitting in grapefruit, and a seedling yellows component that by definition must also be present to cause severe stunting and yellowing of seedlings of Eureka lemon, sour orange, or grapefruit.

The results of the present study showed the existence of tristeza virus strain mixtures, some of which consistently caused stem pitting and others few or no pits on Key lime plants, but there was no indication that the stem-pitting strains from the mildly affected sources was combined with a seedling yellows component. In recent tests, inoculation from the T_0 and T_1 sources has caused no initial symptoms on grapefruit, sour orange, or Eureka lemon. Inoculations from the T_3 source did cause initial shock symptoms of stunting and yellowing on some plants. Under field conditions in Florida, abundant pitting on grapefruit stems infected with tristeza virus has been found in only a very few cases. The relation of the stem-pitting strain of tristeza virus on Key limes in Florida to the strain that causes pitting on grapefruit in other parts of the world remains to be determined.

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DECREASE IN GLYCOLIC ACID OXIDASE ACTIVITY OF WHEAT LEAVES INFECTED WITH PUCCINIA GRAMINIS VAR. TRITICI [1]

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SUMMARY

In 6-day-old wheat seedlings artificially inoculated with *Puccinia graminis* var. *tritici*, the respiratory rate and glycolic acid oxidase activity underwent considerable alterations. In susceptible varieties, a parasitically induced high rise in respiratory rate was correlated with a strong decrease in glycolic acid oxidase activity. In resistant wheats, the respiratory rate was but insignificantly increased and the glycolic acid oxidase activity was only slightly affected. The results are discussed from the point of view of recent developments in the biochemistry of glycolic acid metabolism.

The invasion of higher plant tissues by bacterial or fungal pathogens may be associated with changes in the activity of respiratory enzymes (1, 4, 10, 11, 14). It has been pointed out by the authors of this paper that the parasitically increased respiration of wheat bypasses the Krebs cycle (3) and is mediated largely by ascorbic acid oxidase (5, 6), whereas the normal pathway of respiration in healthy tissues is catalyzed by the cytochrome system linked with succinic dehydrogenase. These findings are in general agreement with the recent results of Shaw,[2] which indicated an increased participation of the direct oxidative pathway (hexose monophosphate shunt) in the respiration of rusted wheat leaves.

The pattern of biological oxidations is, therefore, profoundly altered in the wheat infected with obligate parasites. The present paper gives further information about the changes that take place in the diseased tissues.

Glycolic acid oxidase is an enzyme widely distributed in the plant kingdom (7, 9), and its role in terminal oxidations also has been demonstrated (15). It seemed, therefore, to be of interest to correlate its activity with the characteristic respiratory response in the host tissues induced by infection.

MATERIALS AND METHODS.—Wheat seedlings were grown under ordinary greenhouse conditions and inoculated in the 1-leaf stage with the urediospores of race 21 of the stem rust fungus (*Puccinia graminis* Pers. var. *tritici* (Eriks. & E. Henn.) Guyot). This physiologic race was isolated from a Hungarian population of the rust fungus and identified according to standard methods (13). Two susceptible Hungarian wheats (*Triticum aestivum* L. var. F 481 and R 23) and 3 highly resistant wheat types (*T. timopheevi*, Zhuk., *T. monococcum* L. var. Einkorn, and *T. dicoccum* Schrank var. Vernal) were used throughout the experiments.

The determinations of respiratory rate and glycolic acid oxidase activity were carried out 7–10 days after inoculation. Respiratory rates were followed by means of a Warburg constant-volume respirometer according to the procedures previously described (3).

In the determination of glycolic acid oxidase activity, essentially the procedures of Noll and Burris (9) were followed. One and one-half g freshly harvested wheat leaves was homogenized in 10 ml phosphate buffer (pH 7.8), and 1.5 ml of this crude juice was placed in the main compartment of each Warburg vessel. After equilibration of the temperature, 0.5 ml of 0.1 M lactic acid (dissolved in phosphate buffer and adjusted to pH 7.8) was added from the side bulb. A comparison with blank controls (homogenates without the addition of lactic acid) gave the basis for the determination of Q_{O_2} value ($Q_{O_2} = O_2$ mm³/mg dry matter/hour).

RESULTS.—The mean values of a series of repeated experiments are shown in Table 1. The homogenates from susceptible healthy wheat seedlings were characterized by a fairly high glycolic acid oxidase activity, in strong contrast to the low values found in the juices of diseased plants. It is also evident that the respiratory increase characteristic of susceptible tissues does not parallel the glycolic acid oxidase activity. In resistant wheats (*T. monococcum*, *T. timopheevi*, and *T. dicoccum*), infection resulted in only a slight respiratory response and little change in glycolic

TABLE 1.—*Respiratory rate and glycolic acid oxidase activity in healthy wheat seedlings and in comparable seedlings infected by Puccinia graminis var. tritici*

Wheat variety	Infection type [a]	Respiratory rate (Q_{O_2}) [b]		Glycolic acid oxidase activity [c]	
		Healthy	Diseased	Healthy	Diseased
Triticum aestivum var. F 481	4	2.3	8.3	1.6	0.2
T. aestivum var. R 23	4	2.4	8.5	1.8	0.1
T. timopheevi	1	3.0	4.2	1.5	0.6
T. monococcum var. Einkorn	1	2.6	3.1	1.1	0.6
T. dicoccum var. Vernal	0;	2.6	3.2	1.6	1.1

[a] Infection types are those of Stakman et al (13): 4 = susceptible, 1 = resistant, and 0; = very resistant.

[b] Glycolic acid oxidase activity is also expressed in Q_{O_2} values (increase of O_2-uptake, on the addition of lactic acid to the homogenates, in mm³/mg dry matter/hour).

[c] Oxygen uptake in mm³/mg dry matter/hour.

[1] Accepted for publication November 12, 1956.

[2] Shaw, M. 1956. Personal communication.

acid oxidase activity.

This lack of response in resistant wheats cannot be attributed to a lack of infection, since the tissues were heavily attacked by the fungus and only the hypersensitivity of the cells hindered its further development and fructification. The assays were made during the initial stage of the hypersensitive reaction, i.e. in a stage when the defense mechanism of host had not yet suppressed or eliminated the parasite.

Concerning the mechanism of the inactivation of glycolic acid oxidase in heavily infected susceptible tissues, no definite explanation can be given at present. Extracts from diseased tissues, when added to the homogenates of healthy leaves, did not diminish the enzyme activity. Therefore, the presence of a toxin paralyzing the enzyme in question, like the *in vitro* inactivation by Clostridium-toxins of succinoxidase (8), could not be demonstrated.

Discussion.—The pattern of biological oxidations in wheat tissues infected with obligate parasites undergoes considerable alterations. In the present paper, the discussion centered around the changes in glycolic acid oxidase activity of rust-affected wheat seedlings. It has been pointed out that glycolic acid oxidase activity in diseased tissues is always antiparallel to the respiratory rate. Therefore, the possibility of its participation as a terminal oxidase in the parasitically increased respiration is ruled out. This is in agreement with our previously reported findings on ascorbic acid oxidase as the major enzyme catalyzing terminal oxidations in rust-affected wheat leaves (5, 6).

Stress should be laid also on the observed parallelism between the level of gas exchange of infected plants and their degree of resistance. The highly increased O_2-uptake of rusted susceptible wheat leaves is a well established fact (4, 12). Samborski and Shaw have found recently a moderately enhanced respiration in moderately resistant rusted wheat leaves (12). In the experiments reported herein, the respiratory increase in highly resistant seedlings was fairly small despite the drastic effects of the fungus on the host tissues; infection eventually resulted in the death of great areas of the leaf. This parallelism between the magnitude of the respiratory response and the ability of the fungus to develop and fructify has led us to suggest a positive role of parasitically increased respiration in the development of the fungus (2).

The striking correlation between glycolic acid oxidase activity of diseased tissues and the degree of their resistance makes it tempting to speculate about the possible role of this enzyme in host-parasite relations. It is now generally accepted that the prosthetic group of glycolic acid oxidase is riboflavin phosphate. The vitamin requirements of obligate parasites being highly exacting, a logical working hypothesis seems to be that the pathogen uses the vitamin moiety of the molecule, inducing in this way a fall in enzyme activity.

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CHRONIC DECLINE, A TRISTEZA-LIKE BUD-UNION DISORDER OF ORANGE TREES [1]

Henry Schneider

SUMMARY

Chronic decline, a bud-union disorder of sweet orange trees on sour orange rootstock, causes trees to decline in various degrees. A necrosis of sieve tubes occurs below the bud union and may, when sufficiently extensive, produce typical girdling effects. In most trees, additional anatomical abnormalities that are distinctly different from those of triesteza accompany the necrosis. Excessive numbers of fibers and crystal idioblasts are produced. Immediately below the bud union, the fusiform rays may become much elongated vertically and some become hyperplastic and woody. Bark thickening occurs below but not above the bud union. There was a positive correlation between the amount of functioning phloem and the amount of starch in roots.

A disease known as chronic decline of sweet orange trees on sour orange rootstock has been discovered as a result of intensive research on tristeza disease (formerly known as "quick decline" in California). Tristeza is a serious disease of sweet orange trees on sour orange rootstock and is caused by an aphid-transmitted virus (2, 6). In both tristeza and chronic decline, a necrosis of sieve tubes occurs below the bud union, and the resulting girdling causes trees to deteriorate.

For quarantine and other purposes, accurate diagnoses of tristeza are necessary. In some citrus seedlings, such as the Mexican lime, the tristeza virus causes vein-clearing and wood-pitting symptoms that are specific to the tristeza disease (7). In other citrus plants, such as sweet orange trees on sour orange rootstock, a decline follows infection, but external symptoms are not specific. Because of this lack of specificity, anatomical symptoms of the bud union have been used as an aid in the diagnosis of declining sweet orange trees on sour orange rootstock.

In 1945, the Bureau of Plant Pathology of the California State Department of Agriculture, in conjunction with the various county agricultural commissioners, began making periodic surveys for tristeza. Beginning in 1947, they prepared their own sections for diagnoses and made transmission tests in further confirmation (1). The author was privileged to examine the bud-union sections made from trees suspected of having tristeza. In addition to this, various agencies submitted other material to the author for diagnosis.

Early in 1947, declining trees with necrosis of sieve tubes below the bud union were discovered in Ventura and San Bernardino counties, where tristeza was not then known to occur.: The anatomical characteristics of the bud unions of some of these trees resembled stages found in tristeza-affected trees, but other trees showed stages that were strikingly different and resembled symptoms of chronic decline described herein. Transmission tests involving the latter group of trees, performed by J. M. Wallace and E. C. Calavan, were negative for tristeza.

Anatomical symptoms following the initial onset of necrosis of sieve tubes below the bud union of tristeza-affected trees have been described by Schneider (4), who has also described the anatomy of the trunk of tristeza-free sweet orange (3) and sour orange (5) trees. It is the purpose of the present paper to describe the pathological characteristics of the bud-union anatomy of trees affected by chronic decline and to compare them with those of trees affected by tristeza.

Trees in acute stages of chronic decline have yellow foliage, are partially defoliated, carry small crops of fruit, and are located at random in orchards. Some trees decline in a 1-sided manner.

In most studies on pathological anatomy, we work with plants inoculated with a single virus. Therefore, the anatomical disarrangements are due to the particular virus used for inoculum. In these studies, however, we dealt with a disease, or with several diseases, of unknown cause. Suspect trees were several decades old and had been grown from various budwood sources. Since there are no top symptoms specific to chronic decline, we are attempting to characterize the disease by anatomical symptoms. There is a possibility that not all symptoms observed are those of a single disease.

METHODS AND MATERIALS.—A group of 29 Valencia orange trees, all except 1 on sour orange rootstock, located on the Hinkley property at Bryn Mawr in San Bernardino County, was studied intensively. Among the trees sampled, 2 were in severe decline. Twenty-eight of the trees were in 2 adjacent rows. One additional tree was at the head end of a third row. The head tree of each of the 3 rows was adjacent to an orchard road.

Random samplings were made in other orchards in central and southern California. Also examined were slides prepared by members of the Bureau of Plant Pathology of the California State Department of Agriculture.

In the Hinkley orchard, bark samples for phloem examination were taken from across the bud union

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every 3 months and from 6 in. above and below the bud union and from large roots 18 in. below the bud union at less frequent intervals. Finger-sized roots were dug from near the skirt of the tree, cut with pruning shears, and tested for starch with iodine in potassium iodide solution. The relative quantities of starch in roots were graded on a scale of 0 to 4, based on the intensity of the color reaction. The degree of decline of the tops of the trees was based on an estimate of the amount of defoliation and the degree of yellowing.

Sectioning was done with a freezing microtome and staining was by a procedure using Heidenhain's haematoxylin and lacmoid (3). The maximum number of features may be observed from radial sections across the bud union; however, cross and tangential sections were also made to aid in interpreting what was seen in radial section.

RESULTS.—*Necrotic sieve tubes, amount of phloem remaining functional, and depletion of reserve starch.*—Necrosis of the oldest and sometimes of the middle-aged or even of the youngest sieve tubes occurred below the bud unions of 25 of the 29 trees sampled in the Hinkley orchard on at least part of the sampling dates. This necrosis was followed by degeneration of the older sieve tubes to ¼ in. above the bud union, but no degeneration was present 6 in. above. Necrosis frequently was found from 6 to 18 in. below the bud union.

The 3 trees adjacent to the orchard road were consistently free of necrotic sieve tubes, and the average radial width of their bands of functioning phloem was about 800 μ (Fig. 1). The owner stated that these trees were part of the original planting. Another tree that was consistently free of necrotic sieve tubes was on an odd type of rootstock; chemical tests performed by L. C. Masters and the fact that the rootstock was larger than the scion indicated that it possibly was grapefruit. Since this tree was not on sour orange rootstock, data for it are not illustrated in Figure 1.

Figure 1 shows the amounts of functioning phloem immediately below the bud unions on the various sampling dates for 23 trees free of decline when

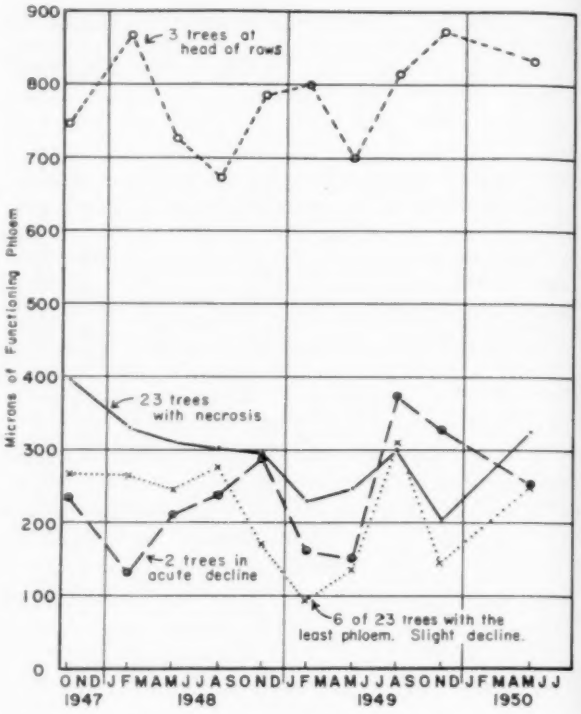

FIG. 1. Radial width on various sampling dates of bands of functioning phloem immediately below the bud union of Valencia orange trees on sour orange stock in the Hinkley orchard, Bryn Mawr, California. No necrosis was present in the 3 trees at the head of the rows.

sampling was begun, for 6 of these 23 trees having the lowest average amount of functioning phloem, and for 2 trees that were in severe decline when sampling was begun.

On some sampling dates, roots of a number of trees gave negative or weakly positive tests for starch. Averages for all the sampling dates for each of the 29 trees showed a correlation (significant at the 1 per cent level) between the average width of functioning phloem and the average amount of starch in the roots. During the sampling, a 1-sided defoliation appeared

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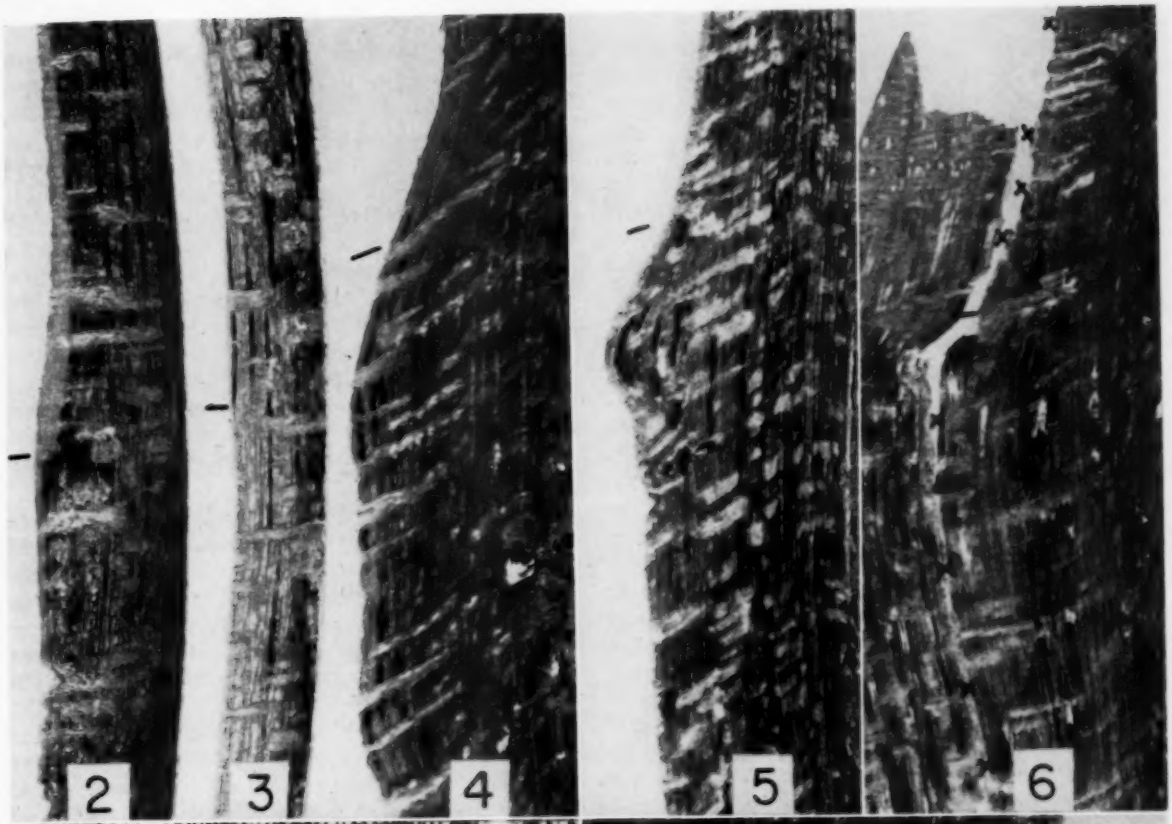
FIG. 2–8. Sections of bark and wood of sweet orange trees on sour orange rootstocks. (Approximate location of bud union in Figures 2–6 is marked by dashes.)—Fig. 2. Radial section of bud union affected by tristeza (× 12). Note swelling above and below. (Fiber bundles represented by inked lines.)—Fig. 3. Radial section of healthy union (× 12). Fig. 4. Radial section of bud union, showing excessive phloem and fiber production for some distance below union of chronic-decline-affected tree (× 12).—Fig. 5. Radial section of bud union, showing localized overproduction of phloem composed mostly of rays, fibers, and idioblasts (× 12).—Fig. 6. Radial section with affected wood partially attached at left (× 12). (Cambial region indicated by *X*.) Excessive numbers of fibers formed during 1 period of phloem production, but more recent phloem was relatively free of fibers. Excessive ray tissue occurs in the region of bud union. Vessels of xylem filled with wound gum. Immediately below the bud union, the xylem is composed of unlignified parenchymatous tissues. In Figures 3–6 the fibers are dense black.—Fig. 7. Tangential section of phloem immediately below bud union (× 120). A, B) Ray higher than normal. C, D) Ray nearly normal. E) Ray higher and wider than normal; central portion pulled out; tissue slightly hyperplastic. n) Necrotic sieve tube.—Fig. 8. Radial section of xylem immediately below bud union (× 120). There are woody hyperplastic rays with protruding portions (W) pulled out from the bark. Number of calcium oxalate crystals excessive (they appear as white beaded areas in vertical rows and oblique rows parallel to rays). Wood is deficient in vessels (V).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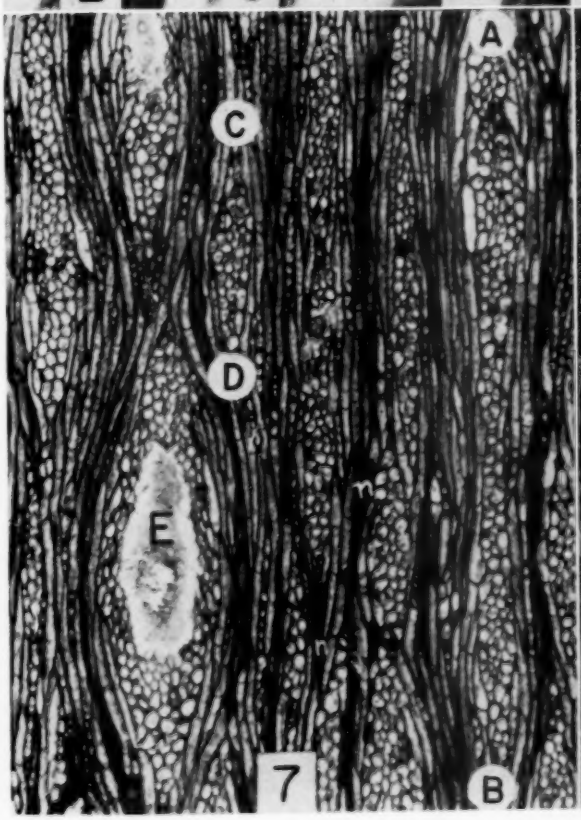
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in 5 trees, but there was no yellowing of foliage.

Abnormalities associated with necrotic sieve tubes.—In addition to necrosis of sieve tubes, other pathological characteristics were present in samples from the Hinkley and other orchards. In the sour orange tissue adjacent to necrotic sieve tubes, some parenchyma cells hypertrophied (Fig. 12) and sometimes were divided by cross walls; however, this condition was not so pronounced as with tristeza. Hyperplasia of ray cells frequently occurred below the bud union (Fig. 7–9, 12). The hyperplastic ray cells were longer and narrower than normal and were pointed rather than rectangular at their ends (Fig. 12). The hyperplastic condition seemed to originate in rays as they were formed at the cambium and not in mature rays that had formed normally. In extreme cases, the cells were reduced from a diameter of about 18 μ to as little as 4 μ, and they became long and fiberlike (Fig. 12). Secondary walls sometimes formed and became lignified. The woody hyperplastic rays extended from the wood across the cambium into the phloem; when the bark was removed, the phloem portion of the rays slipped out of the bark and remained attached to the wood (Fig. 7, 8, 12). This produced a pinhole effect on the cambial face of the bark (Fig. 13) and a hairy appearance on the cambial face of the wood. The fusiform-shaped rays sometimes became joined with others above and below in such a way as to produce excessively high rays (Fig. 7). There was also a tendency for the rays to slant downward from the phloem toward the pith (Fig. 4, 5, 8).

Production of excessive numbers of sheets of fibers in the phloem occurred below the bud union in some trees, but this symptom was not general (Fig. 4–6). The 3 trees at the head ends of rows exhibited no necrosis on 10 sampling dates and averaged 0.7, 1.6, and 1.0 fiber sheets in a 1000-μ band of phloem adjacent to the cambium and at a point 1 mm below the bud union. This was apparently normal for sour orange (5). The 3 trees with the greatest amounts of necrosis but no yellowing of tops averaged 4.5, 3.3, and 1.2 sheets of fibers. This was far above normal in the case of the first 2 trees, but the number of fibers in the third tree was normal in all sections. There was a tendency for excessive fiber production to become localized about the trunk. Occasionally, in groups of sections taken from the same piece of bark, only part of the sections had numerous fibers. In the formation of bark, there were periods of excessive fiber formation and periods when no fibers were produced (Fig. 6). Accompanying excessive fiber formation was a thickening of bark below the bud union. In tristeza-affected bark, thickening occurs a short distance above and below the union at first (Fig. 2), but later it becomes more pronounced below than above.

In chronic decline, there was frequently an overabundance of calcium oxalate crystals in the tissues. In the bark they formed in parenchyma cells both above and below the bud union (Fig. 14) and also below the bud union in the xylem (Fig. 8).

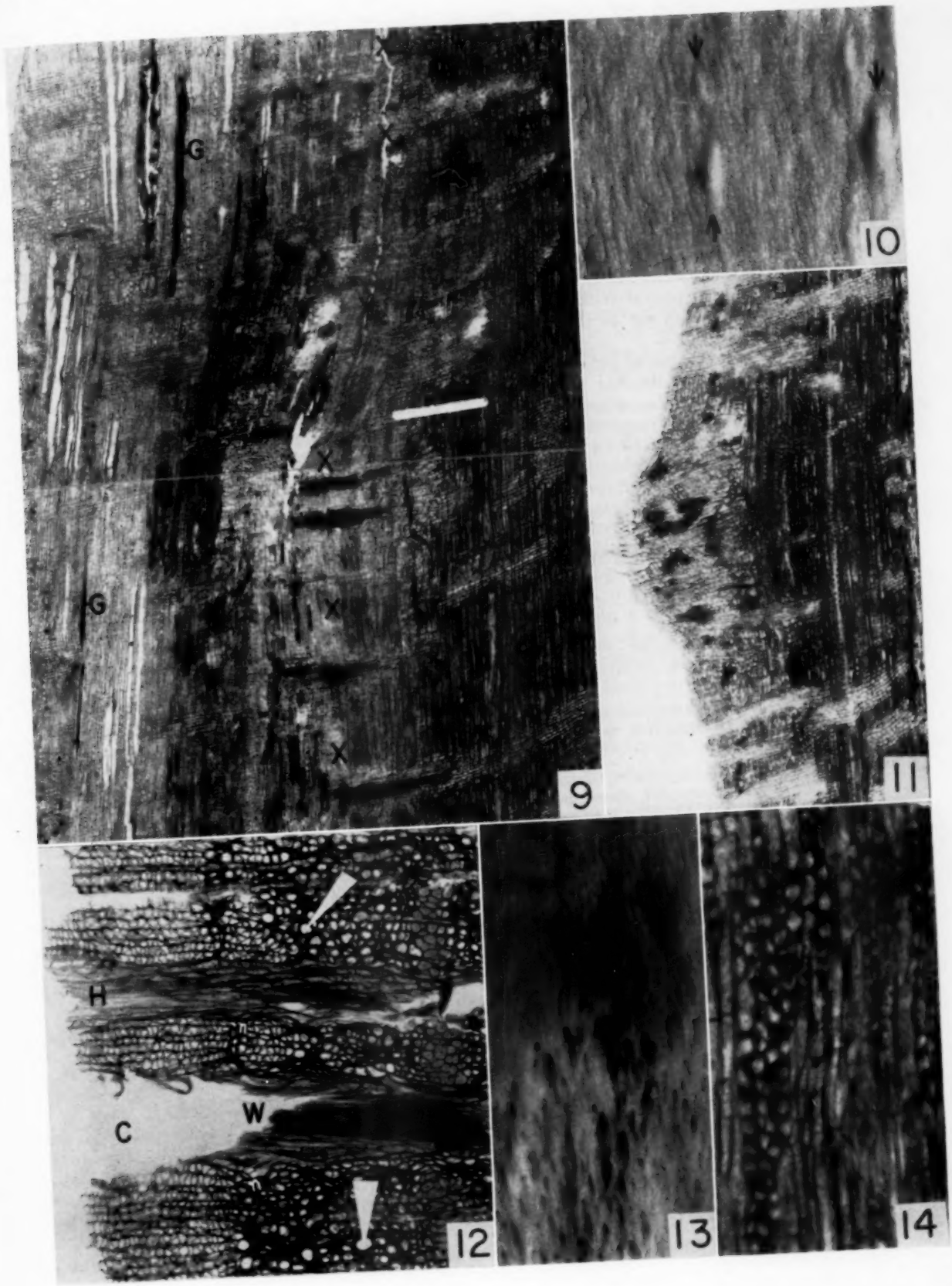
Bulges of abnormal bark composed of rays, fibers, and crystal cells were observed occasionally. These usually occurred immediately below the bud union (Fig. 5).

In extreme cases, the xylem failed to differentiate and lignify just below the bud union (Fig. 6, 9), and there was an excessive number of crystal idioblasts. Hyperplastic rays extended deep into the wood (Fig. 8), and the walls of parts of them failed to become lignified. Affected portions of the xylem were deficient in vessels, and wound gum was sometimes deposited in those present.

Samples were taken 6 in. below the bud union on several of the 10 sampling dates. When necrotic sieve tubes occurred immediately below the bud union, necrosis was present in many instances 6 in. below (Fig. 12). Occasional hyperplasia of rays and hypertrophy of parenchyma accompanied the necrosis (Fig. 12).

In the scion portion of bark samples, pimples (Fig. 10) or the cambial face of the bark and corresponding pitting in the wood occasionally were observed. These pimples were composed of raylike cells and of excessive numbers of fibers and crystal idioblasts, and they either lacked or were deficient in sieve tubes (Fig. 11). It is not known whether or not this symptom belongs to chronic decline. Pimples were only rarely observed in bark samples, but they were more readily found by removing pieces of bark that were larger than those usually taken. They were not present in all trees.

FIG. 9–14. Sections of and cambial faces of bark from sweet orange trees on sour orange rootstock affected by chronic decline.—Fig. 9. Radial section of bud union of severely affected wood and bark from same tree as section shown in 6 (cambial region indicated by *X* and approximate location of bud union by a white line). Some vessels contain wound gum (G). Ray tissue excessive in vicinity of bud union. Hyperplastic rays extend across cambium below bud union. Tissue immediately below bud union is of parenchymatous cells that failed to differentiate into typical xylem and phloem tissue (× 33).—Fig. 10. Swollen areas (pimples) on cambial face of bark above bud union (× 3.5).—Fig. 11. Radial section through area of swollen tissue composed of rays, fibers (dense black areas on left), and crystal idioblasts (× 45).—Fig. 12. Cross section of phloem 6 in. below bud union in severely affected tissue. A hyperplastic ray (H), necrotic sieve tubes (n), hypertrophied parenchyma cells (arrows), and a woody hyperplastic ray (W) are shown. Cavity (C) was left when part of a ray was pulled out (× 120).—Fig. 13. Photograph of cambial face of bark. Oblique cavities occur below bud union where hyperplastic rays were pulled out (× 12).—Fig. 14. Excessive crystal formation in functioning phloem above bud union (× 260).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COMPARISON OF TRISTEZA AND CHRONIC DECLINE CHARACTERISTICS.—As an aid in distinguishing between tristeza and chronic decline, characteristic symptoms of both diseases are recorded in the following sections.

Characteristics of tristeza.—1) Callus tissue, rather than new phloem, occasionally is produced at a point immediately above the bud union. Parts of the callus tissue may become necrotic. In trees in an equilibrium stage, owing to recent phloem formation, such necrotic callus tissue may be located in nonfunctioning phloem at some distance radially from the cambium.

2) There is a characteristic distribution of sieve-plate callus about the bud union in some stages of the disease. The callus occurs above but not below the bud union in phloem present at the onset of the disease; it occurs below but not above the union in recently produced phloem (4).

3) An equal overproduction of phloem occurs immediately above and below the bud union in trees when they first decline (Fig. 2), but more is produced below than above in later stages. Thickening of phloem as a result of disease must be judged by the relation of the contour of the cambium to the contour of tissue present before the onset of the disease. Cells of abnormally thickened phloem are much smaller than normal.

4) Differentiating ray cells sometimes fail to elongate at the bud union.

5) Fibers are lacking or deficient in phloem produced after the onset of necrosis of sieve tubes (Fig. 2).

6) In parenchyma cells below the bud union, globules fail to form as artifacts of fixation with Randolph's modified Navashin's solution.

7) Heavily staining material occurs in some parenchyma cells above the bud union.

Characteristics common to both tristeza and chronic decline.—1) Necrosis of sieve tubes and hypertrophy of parenchyma cells occur below the bud union (Fig. 12).

2) Rays below the bud union become hyperplastic (Fig. 7–9, 12).

Characteristics of chronic decline. 1) Excessive numbers of fiber sheets may develop immediately below the bud union (Fig. 4–6).

2) Ray tissue becomes excessive (Fig. 7, 8).

3) Excessive numbers of calcium oxalate crystals are present (Fig. 8, 14).

4) Protuberances may develop on the cambial face of the bark immediately below the bud union. Such tissue is composed of excessive amounts of ray tissue, excessive numbers of fibers, and idioblasts (Fig. 5).

5) The bark may become abnormally thick below the bud union (Fig. 4–6), but the cells are of normal size.

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REDUCTION OF THE ROOT ROT COMPLEX ON CROFT LILIES BY FUNGICIDAL TREATMENT AND PROPAGATION FROM BULB SCALES[1]

J. G. Bald and Philip A. Chandler

SUMMARY

The unpredictable incidence of root rot on Croft lilies in greenhouses is associated with a complex of pathogens. Before an attempt was made to isolate the causal organisms from the root rot complex, fungicidal treatments were applied to bulbs, bulb scales were removed, and bulbils grown in pots of steamed soil.

A number of bulbils from treated bulbs produced plants free from definite root rot symptoms. Minor toxic damage to roots was associated with a Penicillium (probably a soil inhabitant), with bacteria, and with fungi that were probably clones of *Fusarium oxysporum* Schlecht.

An association between serious damage to individual roots and the production of leaf scorch lesions was confirmed. Apart from the degree of root damage, some Croft lily clones seemed more apt than others to develop leaf scorch lesions.

After nematodes and *Rhizoctonia solani* Kühn were eliminated, *F. oxysporum*, *Pythium ultimum* Trow, and bacteria were isolated from diseased plants. Inoculation tests indicated the occurrence of pathogenic and nonpathogenic clones of *R. solani* and *F. oxysporum*. The 1 clone of *P. ultimum* tested also was pathogenic. Except where mites and nematodes were involved, these 3 organisms appeared mainly responsible for the rotting and discoloration of underground plant parts of field and greenhouse lilies.

A balance seems to exist between *P. ultimum* and *F. oxysporum* as root rot pathogens. In the field, the environment favors Pythium; forcing conditions in the greenhouse are sometimes more favorable for Fusarium.

Bulb scales were surface-sterilized, cultured, and placed in agar tubes. Some were free from detectable organisms. Under aseptic conditions they produced bulbils, which provide a basis for clonal propagation of Croft lilies free from all pathogens except viruses.

A previous study of the disease problem in greenhouse-forced Croft lilies (1) suggested a change from the usual method of investigation, which is initiated by identification of the pathogens involved. Instead, an initial attempt was made to propagate plants free from all symptoms likely to be associated with lesions on underground plant parts. A proportion of plants thus obtained were free from demonstrable root, bulb, and foliar lesions; distinctly pathological symptoms, which appeared on some plants, were generally associated with characteristic root lesions.

Only fungus pathogens of the root and bulb are discussed in this paper, although eradicatory treatments applied to the bulbs were intended to eliminate mites and nematodes. Some Rhyzoglyphid mites are involved in the lily root rot complex and are capable of independent damage to all underground plant parts. Dr. R. N. Jefferson has demonstrated this for 2 species, *Rhizoglyphus rhizophagus* Banks and *R. solani* Ouds, which have been tentatively differentiated from the true bulb mite, *R. echinopus* F. & R. Also, there are several species of nematode that have become the dominant root rot organisms in some lily fields; these are being studied by the Department of Nematology at the University of California.

METHODS.—Twenty-three Croft lily bulbs were cured at 35°C for 1 week, and dried roots were removed. The bulbs were soaked in water for 2 days, then treated with 1 in 200 commercial 40 per cent formaldehyde at 46°C for 2 hours, and finally immersed for 24 hours in a 1:1000 dilution of Puratized Agricultural Spray or PAS (5 per cent tris(2-hydroxyethyl)(phenylmercuri)ammonium lactate)(3). They were later scaled, the scales were treated with excess ferbam (ferric dimethyldithiocarbamate) and kept in trays of moist vermiculite at 21°C until bulbils formed (5). Half of the scales of each bulb lot (clone) were given a second 24-hour dip in 1:1000 PAS before bulbils were removed for planting; the others were not again treated. Clones were lettered and scales numbered, so that every plant could be traced to the scale from which it came. Four lots of bulbils were successively produced and removed from the scales. The supplementary dip with PAS was not again applied. The first 2 lots, 3500 in all, were planted in small pots of steamed U.C.-type soil mix (4).

One hundred forty centers and stem bulblets from the treated bulbs were planted in 6-in. pots.

Bulbil production.—One thousand scales from the 23 bulbs (clones) were used in the propagation trial. There were 50, 40, or 30 scales in a clone. One scale was not recovered at first harvest, reducing the total to 999. Bulbil production varied significantly from one clone to another. The highest production per scale for a single clone was 7.6 bulbils; the lowest was 4.8. The differences seemed to be due more to physiological than to pathological causes. The range of values was 1 to 22 per scale, but 70 per cent of the scales produced 4–7 bulbils.

At first harvest, all scales were turgid and in good condition; at second harvest, a few were incapable of further production. Very little active scale tissue remained at fourth harvest; a scale that had produced its quota of bulbils began to shrink and curl into a mummified remnant. There were differences among

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clones in rate of senescence; these may have arisen in part from microfloral invasion of the senescing tissues, since the differences were practically eliminated from the lots of scales given the second treatment with PAS.

Although the second treatment with PAS helped preserve the scale tissues in most of the 23 clones, it diminished the production of bulbils. Presumably the tissues absorbed sufficient mercury from the dip to affect their metabolism. The growth rates of plants from bulbils directly exposed to the dip (first harvest) and from the bulbils produced 8 weeks later from the same scales (second harvest) were both reduced. In each series, affected plants at first produced narrow leaves that were often laterally curved. Later, leaves were normal, and the plants became indistinguishable from those not given the second PAS treatment.

Development of plants in pots.—Approximately 1500 bulbils were planted singly in small pots between November 24 and 26, 1954, and 2000 between January 18 and 21, 1955. Before the young plants were transferred to 4-in. pots during late March and early April, a few in a number of clones began to lose color (Fig. 1, A), or the lower leaves began to turn yellow (Fig. 1, D); leaf scorch lesions appeared on others (Fig. 1, G).

During the period after transplanting, other plants were sorted out because of foliage symptoms that suggested root infection. Many of those discarded were later found to be healthy, but were not returned to the healthy stock. On May 17, 1955, 6 months after the first planting and 4 after the second, a final sorting was made; afterwards, 1648 of the healthy plants in pots were sent out for further trial, the majority to field-lily growers in Humboldt and Del Norte counties of California and Curry county in Oregon. In addition, 8 of the best plants of each clone were set aside for further treatment and propagation. Altogether, about 2500 of the 3500 plants appeared to be healthy and about 1000 had been discarded; it was estimated that of the 1000, between 500 and 700 had had some real evidence of disease.

No clone was altogether free from plants with foliar and root symptoms of some sort. The proportion of diseased plants in Clone W, in which symptoms appeared very early, was so high that few other than the 8 healthy plants for further propagation could be found. Four other clones were considered too poor to be included in the field trials, but they provided apparently healthy plants for propagation.

Toxic action of microorganisms on roots.—The first roots of bulbil plants were white and completely healthy. As they elongated between the soil mass and pot, some became more or less yellow. A yellow color had been associated with Rhizoctonia mycelium on untreated plants, so a careful examination was made of bulbil root systems. No Rhizoctonia was found. In the moist-chamber environment between the formerly sterilized soil and the pot wall, septate mycelium, unlike that of Rhizoctonia in appearance, was present in contact with the yellowed areas of root. Occasional round, single-celled, slightly verrucose conidia and remnants of Penicillium conidiophores also were found. Germ tubes from the conidia were associated with the same kind of damage to root hairs and root surface as were the mycelial hyphae. Where contact with the side of a hair cell occurred, the wall caved in without rupturing and was yellow-brown in color. The protoplasm underneath was plasmolyzed, but it sometimes appeared almost normal distally and towards the root. More severe damage was followed by death of the hair cell. Similar but less extreme injury was induced in surface cells of the roots, but no penetration of hyphae into the tissues was observed. Similar damage was found in plants grown from the centers of treated bulbs on roots enfolded by the bulb scales. The damage on these roots was caused by a Fusarium, which must have survived the treatments applied to the parent bulbs and scales, that readily formed chlamydospores. Some months later, Fusarium chlamydospores and hyphae were found inside the tissues of discolored or dead roots, but earlier damage occurred without penetration of the tissues. No attempt was made to culture the superficial organisms, but the Fusarium hyphae and chlamydospores were indistinguishable from those of *Fusarium oxysporum* Schlecht. isolated from the rotted bulbs.

Later examination of yellowed areas on roots of bulbil plants in contact with the pot wall revealed external colonies of bacteria, but little or no Penicillium. The abundant growth of Penicillium apparently occurred as a phase in the microfloral succession after partial sterilization of the soil by steam.

Foliar symptoms and root lesions.—On those plants that did not seem continuously healthy, symptoms were of 2 kinds: slight general chlorosis or yellowing of lower leaves and leaf scorch. One type

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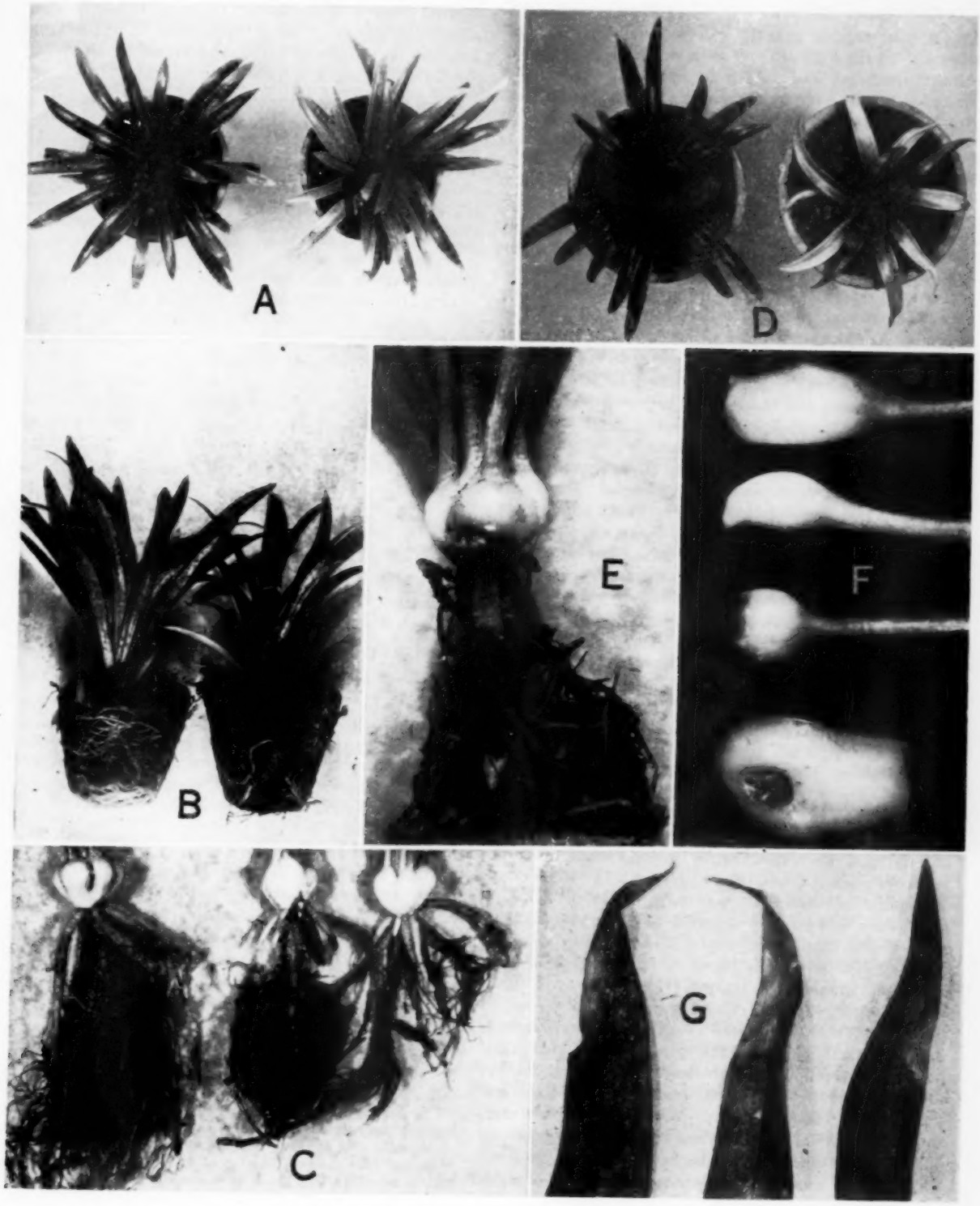
Fig. 1. Bulbil plants of Croft lily propagated from scales of treated bulbs. All were from 4-in. pots of steamed soil. A, B, C) Left: healthy plant; right: plant with root rot, propagated from the same bulb scale. A) Loss of color in leaves of infected plant. B) Undisturbed root systems after the pots were removed. On the diseased plant, few roots were showing; some were discolored or dead. C) Roots after removal of the soil. The diseased plant, having a divided growing point, had produced 2 small bulbs with separate root systems. Main roots were rotted back, finer roots were destroyed. D, E, F) Effects of basal rot on a bulbil plant. D) Diseased plant right, beside a comparable healthy check. E) The same plant (1.5×) with rotting basal plate and rotting bases of bulb scales and roots. F) Four outer scales of same plant, serious lesions on 2 scales. G) Left and center: 3 leaves (1.5×) with advanced leaf scorch lesions involving tips. Right: a lesion beginning on the right side of the leaf.

or both might appear on a single plant.

In many cases where yellowing was confined to 1 or 2 basal leaves, recently emerged roots had ruptured the tissues connecting leaf and stem. In some instances there was evidence of physical damage during repotting. Occasionally root lesions were present.

In instances of continuing increase in number of basal yellowed leaves or a general slight chlorosis, growth was stunted and root lesions were present. Lesions that occurred in numbers over an area of root



surface were small, discrete, and more or less sunken; they were not greatly elongated. The efficiency of the roots was reduced by destruction of root hairs. More serious root damage and concomitant leaf yellowing existed when the lesions extended, and a number of roots were girdled or rotted back from the tips (Fig. 1, C).

At this stage of growth, lesions generally were not present on the bulb scales; when they were, they were few, superficial, and golden to rusty-yellow in color. Similar lesions were found occasionally on the petioles of lower leaves. In a very few instances (3 in 3500) at this stage of growth, typical Fusarium basal rot (2) occurred (Fig. 1, D, E, F).

Leaf scorch lesions on small plants were relatively large (Fig. 1, G), but there were seldom more than 1 or 2 on a plant. On about 70 per cent of the affected plants examined the association between typical leaf scorch and lesions on 1 or 2 roots below (1) was evident. Physical injury during repotting could have accounted for some, but not all, of the remaining leaf scorch lesions.

On bulbil plants, there were no observable differences between root lesions associated with basal yellowing of leaves and those associated with leaf scorch or with a combination of both symptoms. The difference appeared to be in the number and distribution of lesions. Lesions that destroyed 1 or 2 contractile roots on 1 side of a plant were likely to be associated with leaf scorch lesions above; where many roots (including the smaller branches) were attacked, the symptoms were likely to be a yellowing of leaves that began at the base and a stunting of growth. These sometimes were accompanied by leaf scorch.

An experiment was conducted in the greenhouse on the induction of leaf scorch lesions by injury of apparently healthy root systems, but no lesions appeared. Temperature and light intensity were high and humidity low. Injury of diseased root systems of plants inoculated under similar conditions with *Fusarium oxysporum* increased the numbers of leaf scorch lesions. Later, lesions on 2 pairs of apparently pathogen-free plants were associated with root injury caused by tapping plants out of the pots. These plants were in pairs from 2 clones. They were grown from bulbils that developed under aseptic conditions in tubes of agar. These were planted in 4-in. pots. One plant of each pair was tapped out of the pot about once a week for a period of 2 months. Roots were visibly damaged; about 4 days later, 1 to 3 lesions appeared on the leaves of treated plants. The maximum numbers of lesions evident at any 1 time were 7 on 1 plant and 13 on the other. There were no lesions on the untreated checks. The environment in the chamber where the plants were kept was partially controlled. The air was filtered, the humidity ranged between 60 and 100 per cent, and light intensity was relatively low. Minimum temperatures were 18° to 21°C, and maxima were generally 24° to 30° but sometimes a little higher.

CULTURING AND INOCULATION TESTS.—Cultures from discolored bulb scales were made by Mr. R. D. Durbin, using a technique of washing, fragmentation of the tissues, and serial dilution in place of surface sterilization. Five representative colonies of *Rhizoctonia solani* Kühn were selected from hyphal tip subcultures. Also chosen was a culture of a Fusarium derived from a single spore; this was the only other significant genus of fungi appearing in appreciable numbers on the plates. It was identified by Dr. W. C. Snyder as *Fusarium oxysporum* Schlecht.

From many diseased roots (Fig. 1, A,B,C) and from basal rot lesions (Fig. 1, D,E,F) on scales of bulbil plants, apparently the same Fusarium was obtained. Two isolates from the latter source, which were derived from single spores and chosen for inoculation trials, also were identified by Dr. Snyder as *F. oxysporum*.

In some root pieces that had been cultured on potato-dextrose agar and on plain agar, microscopic examination after incubation revealed a Phycomycete, even though no Phycomycete mycelium was observed then or earlier on the agar. Examination of some rotting roots revealed active nonseptate mycelium a considerable distance behind the advancing margin of the lesions. After several failures, a culture of the Phycomycete was obtained from 1 of 30 root pieces cultured singly in plates of plain agar. Subcultures of the isolate were contaminated with an unidentified bacterium, but they were used in that condition in the inoculation of lily plants (Table 1). A culture was freed from the bacterium by an antibiotic, and the bacterium was cultured separately. The 2 organisms were applied, individually and combined, in a second inoculation trial. The Phycomycete was identified by Dr. John Middleton as *Pythium ultimum* Trow.

INOCULATION TESTS.—Table 1 summarizes the results of a series of inoculation trials. Inoculations

TABLE 1.—*Number of Croft lily plants infected and number of lesions on scales and roots of such plants following inoculation with selected cultures isolated from tissues of diseased Croft lilies*

Inoculum		No. of plants infected[a]		Lesion formation[b] on inoculated plants	
Species	Culture	Inoculated	Check	Scales	Roots
Rhizoctonia solani	15	0/3	0/2	—	—
	16	3/3	0/2	+	?
	17	2/3	0/2	+	?
	22	0/3	0/2	—	—
	23	0/3	0/2	—	—
Fusarium oxysporum	(a)	0/5	0/3	—	—
	(b)	5/5	0/3	+	+
	(c)	5/5	0/3	+	+
Pythium ultimum		13/13	0/10	—	+

[a] Numerator indicates number of plants infected and the denominator the number of plants used.

[b] A plus sign indicates a large and consistent difference between inoculated plants and checks in number and type of lesions. In addition it indicates that sampled lesions, examined microscopically, contained fruiting structures and/or mycelium like those of the organism in the inoculum.

were made during the repotting of bulbil lily plants from 2½-in. into 4- or 5-in. pots. Inocula of the Rhizoctonias and Fusaria consisted of mycelium, with a minimum of agar, from tube cultures. Two small pieces of inoculum were placed in the fresh soil added to each pot; 1 piece was placed ¼ to ½ in. from the bulb and the other ¼ to ½ in. from the bottom of the root mass. Inoculations with the subculture of *P. ultimum* that was contaminated with a bacterium and that was bearing zoosporangia and oospores were made by minimal shredding of the mycelium in water and gently rubbing the roots of bulbil plants, shaken free from soil, in 5 ml of the suspension. The roots of 3 other plants were totally immersed in a beaker of inoculum.

When the plants were examined at the end of the experiment, none of the checks had severe lesions, but some of the finer roots of the check plants were not entirely free from damage produced by toxic action of the microflora.

Two strains of Rhizoctonia were pathogenic; they caused yellow discoloration and superficial damage to the bulb scales and probably an increase of toxic damage on the roots. The 2 isolates of Fusarium from the plant with basal rot and root rot produced the full range of symptoms on all inoculated plants. Consequently, they may be called *F. oxysporum* f. *lilii* Snyd. & Hans. The third Fusarium gave no evidence of pathogenicity. It appears to be an undifferentiated clone of *F. oxysporum*. Although *P. ultimum* appeared uniformly pathogenic from the record of lesions in Table 1, symptoms varied greatly from plant to plant, both in type and incidence on main and lateral roots. On some plants, symptoms were very severe; on others they were relatively mild. Healthy young roots grew out between the dead and damaged ones. *P. ultimum* appeared under the conditions of the trial to be a somewhat weaker or less consistent pathogen than *F. oxysporum*. In another trial, inoculation with the bacterium alone provided no evidence of pathogenicity; in combination with the *P. ultimum* there was slight but not consistent evidence that it increased the severity of symptoms on the roots. Inoculations with *P. ultimum* alone also produced root rot; symptoms were like those on some plants inoculated with the original cultures or with artificially contaminated cultures.

An attempt to ascribe the various root rot symptoms to particular organisms might be made from the inoculation trials, but it would appear premature to do so. Comparison between inoculated and naturally infected plants showed that discrete reddish lesions of various sizes may be associated with Fusarium; a rotting back of roots from the tip towards the base may also be associated with Fusarium but might be due to Pythium with or without other organisms. Much of the cortical rot on roots of various sizes, which was often followed by complete collapse, appeared to be associated with Pythium, but Fusarium chlamydospores and mycelium were found frequently in the diseased tissues.

Incipient rotting in the basal plate of the bulb, without penetration of the scale tissue, has been associated with Fusarium in greenhouse-grown bulbs, but abundant oospores and chlamydospores of *P. ultimum* have been found in similar incipient lesions on field-grown bulbs from northern California. Sometimes there were numerous bacteria but few or no fungus hyphae on the surface of discolored scales, intercellularly at the advancing edge of lesions, or in vessels more or less damaged and clogged with gum.

Among so many possible pathogens, which in untreated stocks would probably include Rhizoctonia and nematodes, there is likely to be considerable overlapping of symptoms and chances for interaction. No stable symptom picture is likely to exist on field-grown Croft lilies and their greenhouse progeny.

DEVELOPMENT OF ROOT AND BULB DISEASE IN THE FIELD AND GREENHOUSE.—In the fields of northern California where Croft lilies are grown, many young plants have deficient basal root systems, and thus cannot grow very actively until stem roots develop. Some outer scales rot rather than become senescent; on the surface of the outer bulb scales, there appears the yellow color that has been considered an evidence of maturity, but which is perhaps nearly always evidence of pathogenic injury. Otherwise, bulbs in the better fields appear sound.

The balance of organisms in the root rot complex under field conditions suggests considerable activity by *Pythium ultimum*. Transfer of bulbs to warm conditions in a greenhouse favors Fusarium and reduces the relative importance of Pythium. Rhizoctonia grows actively over the surface of underground plant parts both in the field and the greenhouse, but an estimate of its relative pathogenic effects so far has been impossible.

Vigorous roots emerge after one of the better lots of Croft lily bulbs is received for forcing, planted in pots, and grown in the greenhouse; however, sooner or later root lesions appear, and growth and flowering of some plants may be seriously affected. Eventually Fusarium basal rot of bulbs begins on a few plants, but sometimes this does not occur unless the bulbs are matured and regrown in the greenhouse. As the plants in pots are normally sold by the forcer before the flowers open, rotten or unthrifty root systems may be the only symptom noticed. If the bulbs are from a badly infected field crop, destruction of roots, basal rot of bulbs, and aboveground symptoms are more severe and appear more rapidly on a greater number of plants than when bulbs are from one of the better fields.

The most interesting among a series of Croft lily plants grown 2 seasons in the greenhouse was a group transferred, before flowering the second year, to a lathhouse. The plants matured there in cool autumn weather. About half the plants had bulbs with basal rot, and the apparently sound bulbs had strongly yellowed outer scales. Superficial paradermal sections of yellowed areas carried all components of the pathological microflora discussed above. *P. ultimum* was

abundant in remnants of decayed bulb scales. Another fungus frequently was seen on the surface of yellowed bulbs, but it has not yet been recognized in culture. It has fine superficial mycelium and simple septate, slightly pigmented conidiophores, each arising from a strongly developed foot cell and exuding tiny hyaline oval phialospores. These plants were not from the same source as the 23 bulbs that originated the clones submitted to the schedule of treatments described at the beginning of this paper.

PATHOGEN-FREE CROFT LILIES.—The treatment schedule used in this work was compounded from separate trials of fungicidal dips and hot-water treatments. It was applied to reduce infection and to narrow the field of inquiry. It is not yet recommended for use on any other basis. There is insufficient evidence to show whether any slight alterations or slightly altered conditions would destroy its effectiveness. Nevertheless, it has been applied with several modifications of the original schedule and has given good control of root rot. Some plants appear to have been pathogen free. Other techniques for obtaining pathogen-free stocks of lilies also have shown promise. Washing bulb scales or bulbs in turbulent running water has been a valuable preliminary to fungicidal treatment. In an early trial in which bulb scales were washed for 2 days and then surface sterilized in a 1:20 dilution of 5 per cent commercial hypochlorite solution, about 10 per cent of them were freed of pathogens. Maintained on agar slants in test tubes, 2 of the scales produced 4 sterile bulbil plants.

The following was the first definitive experiment on the production of bulbils from aseptic lily scales. A bulb with bulblets, produced in the greenhouse from a treated clonal stock of Croft lily, was harvested. It was washed in turbulent running water, first after cutting off the stem and most of the roots, then after separating 2 bulbs and 4 bulblets, and again after separating it into scales—altogether 3 days. There were a few lesions on the roots and slight invasion of the basal plates; the extreme tips of some scales were dead. Except on the outside of the bulb and bulblets, the surface of the scales was white and unblemished. The scales were divided into 2 lots, those without any blemish and those either with dead tips or with a slight browning at the base that represented intrusion of organisms from the basal plate. Of the unblemished lot, 14 were surface sterilized with a 1:12 dilution of a 5 per cent commercial hypochlorite solution and plated on potato-dextrose agar. Fusarium grew from the base of 4 scales, bacteria appeared on a fifth, and 9 remained sterile. Of 15 with slight lesions or dead tips, 8 produced fungal growth (mainly Fusarium) from the tips, 5 from the base, and 2 remained sterile.

In later lots of cultured scales, totalling about 300, the infestation by fungi was further reduced by cutting off all tips except those quite free from damage and eliminating all scales with any suggestion of other lesions. There were variations in proportion of bacterial contamination according to the thoroughness of washing and the condition of the air in the culture room. In every case, a long period of washing appeared to be a valuable preliminary to surface sterilization.

Application of a "cultured-scale" technique to obtain pathogen-free plants now appears possible. It would differ from cultured-cutting techniques in that whole scales might be tested and kept under sterile conditions until the bulbils had formed. The rate of growth and propagation of Easter lilies is such that relatively few pathogen-free plants in pathogen-free soil could form the basis of an extensive multiplication program.

DISCUSSION.—Questions of balance and interaction of the organisms in the root rot complex may exist at 2 levels, interspecific and intraspecific. Both pathogenic and nonpathogenic clones of *F. oxysporum* and *R. solani*, and probably clones of intermediate pathogenicity, are present on Croft lily. It is a common rule of biology that intraspecific competition is very severe. This may help to explain why types of *F. oxysporum* that cause lily basal rot attack the bulbs so slowly after transfer from field to greenhouse. Root rot lesions associated with Fusarium are present far more often than are basal rot lesions on the better stocks of field-grown lily bulbs. Also, root rot develops rapidly in the greenhouse. It seems likely that some root-rotting types of Fusarium are unable or are barely able to cause basal rot of bulbs. The tendency of growers to discard rotting bulbs and the natural intraspecific competition will lower and disperse the concentration of basal rot inoculum to a point where it is ineffectual in the field; even in the warmer greenhouse environment, it becomes effective slowly.

The problem of leaf scorch and its causes (1) again appeared in this work. The influence of pathological factors in its incidence was indicated; however, like the better-known influence of nutritional factors (7, 8), the pathological effects were subject to unexplained deviations. A suggestive set of data was obtained from part of the clonal material grown to maturity in a commercial greenhouse under the care of Mr. R. H. Sciaroni. Leaf scorch developed; overall, the numbers of lesions on individual plants were directly related to the severity of root rot. Root rot was judged by the condition of the undisturbed root system tapped out of the pot. In spite of the general agreement, some clones, irrespective of the average condition of their roots, were more apt than others to have leaf scorch lesions. Such differences might be based on inherent characters of the clones. Alternatively, they might be a consequence of differences among clones in strains of a latent virus or of differences in incidence of root-rotting organisms.

Leaf scorch lesions have now been produced by physical injury of the roots on 2 plants from bulbils developing under aseptic conditions. It is at least possible, however, that organisms, particularly bacteria, could have persisted within the scales from which the bulbils arose without invading the agar in

the culture tube. Experiments with larger numbers will be necessary to rule out this possibility.

After this paper was written, a paper by Scheffer & Haney (6) giving the results of trials on the control of *Rhizoctonia solani* and *Pythium ultimum* was published. Lily and poinsettia plants were grown in pots of infested soil and fungicidal treatments were applied to bulbs. Established fungicides failed to control Pythium, which seemed to be the main pathogen. As Fusarium also is difficult to control by these means, it would seem advisable to continue work on application of culturing techniques in the production of pathogen-free lilies.

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THE SYSTEMIC ACTIVITY OF CYCLOHEXIMIDE IN WHEAT SEEDLINGS[1]

V. R. Wallen and R. L. Millar[2]

SUMMARY

The systemic activity of cycloheximide in wheat seedlings was demonstrated by a bioassay method following applications of the antibiotic to the leaves or to the substrate in which the seedlings were growing. Cycloheximide applied as a spray to the leaves was absorbed and could be recovered from the leaf tissue for at least 5 weeks after application, depending on the concentration of antibiotic applied to the seedlings. When cycloheximide was supplied to the roots of seedlings growing in either quartz sand or soil, the antibiotic was absorbed by the roots and translocated throughout the stem and leaf tissues. Translocation of the antibiotic into tissues produced subsequent to the application of the antibiotic could not be demonstrated. The amount of antibiotic absorbed by the seedlings depended on the concentration of cycloheximide supplied to the roots.

Paper chromatography of the active material contained in chloroform extracts and guttation fluid obtained from cycloheximide-treated plants yielded Rf values similar to the values obtained for pure solutions of the antibiotic.

The effectiveness of antibiotics in controlling plant disease has stimulated considerable interest in the possibility that these materials may act systemically to effect a protective or an eradicative action against plant pathogens. Brian (1) applied griseofulvin to wheat seedlings growing in a nutrient solution. A bioassay of the guttation fluid demonstrated the presence of a fungicidal material in the guttation drops within 2–3 days of the application of the antibiotic. Mitchell and co-workers (4) were successful in obtaining evidence of the upward translocation of streptomycin sulfate following applications of the antibiotic to the stems and leaves of bean plants. Similarly, Pramer (5) found that Chloromycetin (chloramphenicol) and streptomycin are absorbed and translocated in cucumber seedlings. Paper chromatography was employed to characterize the active materials recovered from the plant tissue. Hamilton and co-workers (2) were unable to demonstrate any

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translocation of cycloheximide from sprayed to unsprayed cherry leaves; however, cycloheximide derivatives were translocated from sprayed to unsprayed leaves in such concentration as to cause a significant decrease in the number of lesions produced by the cherry leaf spot fungus.

In a previous publication, Wallen (6) reported that cycloheximide (3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide) was effective in controlling stem rust of wheat in greenhouse and field trials. The degree of control obtained with a postinfection spray of cycloheximide suggested a systemic action by the antibiotic. This paper presents the results of an investigation of the systemic activity of cycloheximide in wheat seedlings following applications of the antibiotic to the leaves and to the roots.

METHODS AND RESULTS.—*General Methods.*—A bioassay method was employed to measure the antibiotic activity in seedlings of Garnet wheat following applications of cycloheximide to the leaves or to the substrate in which the seedlings were growing. Excised leaves and chloroform extracts of both treated and untreated plants were assayed in Petri plates on an agar medium seeded with *Saccharomyces pastorianus* Hansen.

The assay method of Whiffen (7) was adapted to the present investigation. The assay medium was prepared in test tubes, each containing 18 ml, and stored at 5°C. Prior to preparation of the plates for assay, the medium was cooled to approximately 45°C; 1-ml volumes each of a solution (0.06 per cent w/v) of streptomycin sulfate and of a suspension of cells of *S. pastorianus* were added to each tube. The addition of streptomycin to the medium inhibited the growth of bacterial contaminants from the plant tissue, without affecting the growth of the test organism. The resulting suspensions were added to individual sterile Petri plates.

Large quantities of cells of *S. pastorianus* were obtained by growing the organism in shake culture. The cultures were diluted with phosphate buffer to a concentration of 200,000 cells per ml and stored under refrigeration.

Two procedures were followed in assaying the plants for antibiotic activity. The plants were excised at a point on the stem just above the soil level and sterilized by exposure to propylene oxide fumes for 24 hours. They were then transferred to the surface of agar seeded with the test organism. A second procedure consisted of grinding excised plants either singly or in groups of 5 in 1 ml of chloroform in a tissue grinder. Filter paper discs (Schleicher and Schuell, No. 740-E) were immersed in the chloroform extract until saturated, and these were then air dried and transferred to the surface of agar freshly seeded with the test organism.

After an incubation period of 24 hours at 30°C, the plates were observed for inhibition in growth of the test organism. The antibiotic activity of the extracts or the plant material was recorded in terms of the size (mm) of the inhibition zone that was evident as a clear area of agar around the filter paper discs or plant tissue.

TABLE 1.—*Antibiotic activity of chloroform extracts of wheat plants sprayed with various concentrations of cycloheximide, as indicated by zones of inhibition on agar seeded with Saccharomyces pastorianus*

Concentration (ppm) of cycloheximide	Inhibition zones (mm) produced by extracts of single plants (A) and of lots of 5 plants (B) after indicated interval							
	1 day		7 days		14 days		21 days	
	A[a]	B[b]	A	B	A	B	A	B
0	0	0	0	0	0	0	0	0
25	5	0	0	0	0	0	0	0
50	19	20	4	0	12	0	0	0
100	4	22	14	33	26	34	0	26
200	32	32	26	30	32	32	9	18
500	35	34	38	40	35	36	30	35

[a] Average diameter (mm) of 4 replications.
[b] Average diameter (mm) of 2 replications.

Leaf absorption of cycloheximide.—Garnet wheat was sown at the rate of 100 seeds per flat in composted soil. When the seedlings were approximately 3 in. high, they were sprayed with solutions of 25, 50, 100, 200, and 500 ppm cycloheximide. At intervals of 1, 7, 14, and 21 days after spraying, the plants were excised and washed in water to remove cycloheximide from the surface of the plant material. The plants then were ground both singly and in lots of 5 in chloroform, and the chloroform extracts were assayed for antibiotic activity. Four single-plant extracts and two 5-plant extracts were prepared from the materials sprayed with each of the cycloheximide concentrations and were assayed. An assay plate containing a single filter paper disc was used for each extract.

The application of cycloheximide as a spray to the leaves of wheat seedlings resulted in an absorption of the antibiotic by the plant tissues (Table 1). Plants sprayed with cycloheximide at concentrations as low as 50 ppm yielded chloroform extracts having marked antibiotic activity. Moreover, in a separate experiment, active material was recovered from the plant tissues for at least 5 weeks after the plants were sprayed with the higher concentrations of cycloheximide.

Individual plants varied in their absorption of cycloheximide. This was apparent when the antibiotic activities of single-plant extracts from plants sprayed with 50 and 100 ppm cycloheximide were compared. Zones of inhibition ranging from a trace to 22 mm were obtained from replications of a single treatment. In spite of this variability, good agreement was obtained when the means of inhibition zones for both extraction procedures were compared.

Root absorption of cycloheximide.—Wheat seedlings grown in quartz sand in the greenhouse were supplied with Hoagland's solution (3) until 1 week after they had emerged. Following this period, Hoagland's solution containing cycloheximide at concentrations of

25, 50, 100, 200, and 500 ppm was supplied to the plants at 2-day intervals for 8 days. Twenty-four hours after the final application of cycloheximide, the seedlings were excised and treated with propylene oxide. The plants then were assayed for antibiotic activity by transferring them to Petri plates containing seeded assay agar.

The degree of antibiotic activity exhibited by plants treated with cycloheximide depended on the concentration of antibiotic supplied to the plants; a gradual increase in zone size occurred with each increase in concentration of cycloheximide (Fig. 1). The systemic nature of the antibiotic material is clearly indicated in Figure 1.

In a similar experiment, the concentration of antibiotic in plants supplied with various concentrations of cycloheximide was estimated by the following method. The active substance from individual plants was extracted in 1 ml of chloroform and assayed as described above. Known concentrations of cycloheximide also were prepared, and these were assayed at the same time.

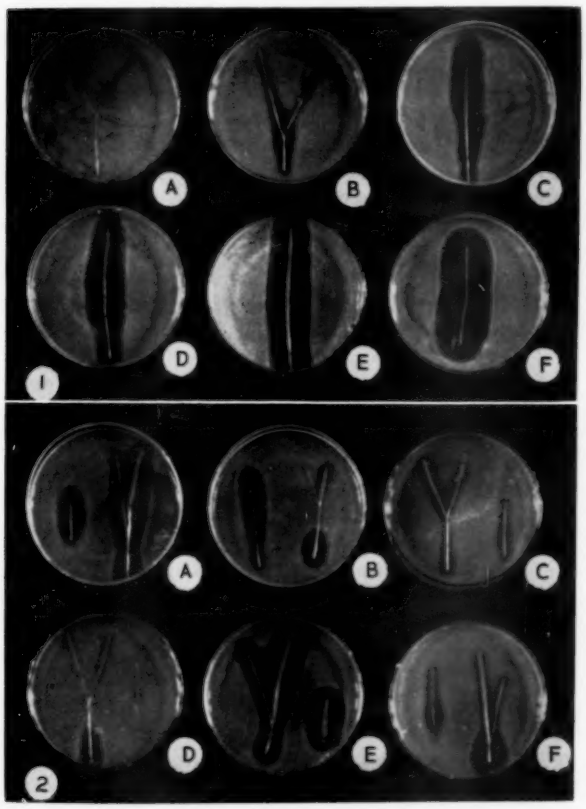

FIG. 1-2. Fig. 1. Inhibition zones produced by wheat seedlings grown in quartz sand and supplied with various concentrations of cycloheximide. The treatments, with widths of inhibition zones in parentheses, were as follows: A) check (0), B) 25 ppm (trace), C) 50 ppm (18±2 mm), D) 100 ppm (21±2 mm), E) 200 ppm (23±2 mm), and F) 500 ppm (31±2 mm).—Fig. 2. Inhibition zones produced by wheat seedlings grown in 3 soil types containing 500 ppm cycloheximide. A,D) Light sand. B,E) Sandy loam. C,F) Clay.

TABLE 2.—*Antibiotic activity of control concentrations of cycloheximide and of chloroform extracts from plants supplied with the same concentrations of cycloheximide*

Concentration (ppm) of cycloheximide in controls and in solutions supplied to plants	Width in mm of inhibition zone[a] on agar seeded with *Saccharomyces pastorianus*	
	Controls	Plant extracts
0	0	0
1	17 (±1)	0
5	27 (±2)	0
10	31 (±2)	0
20	35 (±2)	0
25	37 (±1)	trace
50	40 (±2)	18 (±1)
100	41 (±2)	21 (±5)
200	46 (±1)	29 (±3)
500	50	33 (±3)

[a] Averages of 4 replications.

The inhibition zone produced by chloroform extracts from plants supplied with cycloheximide at 100 ppm was slightly larger than the inhibition zone produced by a 1-ppm solution of cycloheximide in chloroform (Table 2). It was assumed, therefore, that there were at least 1, 5, and 10 μg of active material in plants supplied with 100, 200, and 500 ppm of cycloheximide, respectively. Calculated on the basis of the dry weights of plants in each treatment, the concentrations of active substance in plants supplied with 100, 200, and 500 ppm cycloheximide were 70, 590, and 1000 ppm, respectively.

The absorption of cycloheximide from soil also was investigated. Garnet wheat seed was sown in rows in flats of 3 types of soil: a light sand, a sandy loam, and heavy clay. After the seedlings emerged, furrows were made between the rows and 50 ml of a 500-ppm solution of cycloheximide was poured into each furrow. The cycloheximide solution was added every 2 days for 8 days and the plants were harvested 24 hours after the final application of antibiotic. Four plants from each soil type were treated with propylene oxide and transferred to seeded assay agar. In addition, 4 plants from each soil type were extracted with chloroform.

Cycloheximide was absorbed by plants in each soil type. Zones (Fig. 2) varied from a trace to 33 mm; there was no apparent effect of soil type on absorption of the antibiotic by plants. The variation in size and distribution of the inhibition zones suggests that the amount of antibiotic absorbed varied considerably with individual plants. In certain plates, an inhibition zone was apparent around the entire plant, whereas in other plates the test organism was inhibited by only a part of the plant.

An experiment employing the same conditions was performed to determine how long plants retained antibiotic activity. For this experiment, only chloroform extracts of plants were assayed. Extracts of single plants were assayed for activity at weekly intervals for a period of 7 weeks after the final application

of antibiotic.

Throughout the entire period, extracts that inhibited growth of the test organism were obtained. There was marked variation, however, in the degrees of inhibition produced by replications of a treatment. Zones of inhibition ranging from 0 to 30 mm were recorded. This marked variability probably was caused by an irregular movement of the cycloheximide solution from the furrows through the soil to the plants.

The data obtained from the foregoing experiments indicate that cycloheximide is absorbed through the roots and becomes distributed throughout the plant tissues already formed at the time the antibiotic is applied to the medium in which the plants are growing. The following experiments were performed to determine if there is a movement of the antibiotic from such tissues into plant tissue developing subsequent to the application of the antibiotic.

Wheat seedlings growing in quartz sand were supplied with a 200-ppm solution of cycloheximide according to the procedure described above. Twenty-four hours after the final application of antibiotic, the seedlings were removed from the crocks; the roots were washed thoroughly with water, and the plants then were transplanted to greenhouse soil. The seedlings were assayed for antibiotic activity 13 days after their transfer to the soil medium. No activity could be detected in any of the plant tissue produced subsequent to the final application of antibiotic. Tissues formed prior to the application of antibiotic, however, produced large zones of inhibition.

The 3 soil types indicated above were employed to determine the extent of movement of antibiotic into new plant growth. Garnet wheat seed was sown in each type of soil in wire-mesh baskets. When the seedlings had reached the 2-leaf stage, the baskets were transferred to trays containing a 50-ppm cycloheximide solution. Because of the procedure employed, solutions at concentrations higher than 50 ppm were markedly phytotoxic to the seedlings. The baskets were kept in the antibiotic solution until the soil was saturated, and then they were moved to the greenhouse bench. After 24 hours, plants in each soil type were excised at the soil level, sterilized with propylene oxide, and plated directly on assay agar. Antibiotic activity was demonstrated for plants from all soil types. Additional plants were assayed when the seedlings had reached the third-leaf stage. Zones of inhibtion were produced only by leaves formed prior to the application of the antibiotic. Moreover, the inhibition zones were located only at the tips of the leaves; the remainder of each seedling did not exhibit any activity.

Paper chromatography.—Paper chromatography was employed to characterize the active material present in plants grown in quartz sand and supplied with Hoagland's solution containing 500 ppm cycloheximide. Following extraction of the active substance with chloroform, the plant extract was compared with a solution of cycloheximide in chloroform by means of descending paper chromatography. The solutions were spotted on strips of Whatman No. 1 filter paper. Molar phosphate buffer at pH 6.8 was used as the developing solvent. After their development in the chamber at a temperature of 22°C, the strips were air dried and tranferred to the surface of agar seeded with *S. pastorianus*. Following an incubation period of 24 hours, inhibition zones were located and the Rf values of both the active substance in plant extracts and the pure antibiotic calculated. The values obtained for these preparations were 0.61 and 0.59, respectively.

The same procedure was followed to compare the guttation fluid obtained from cycloheximide-treated seedlings and an aqueous solution of cycloheximide. Identical Rf values (0.59) were obtained for both preparations.

The above data support the tentative conclusion that the active material present in plant extracts is either cycloheximide or a closely related compound.

Discussion.—Data presented above indicate that cycloheximide is absorbed by the leaves and that absorption of the antibiotic through the roots is followed by its translocation throughout the plant tissues present when the antibiotic is applied. Studies conducted in 1954 (6) showed that a single spray application of cycloheximide at a concentration of 100 ppm successfully controlled stem rust of wheat. Based upon our findings in this paper, a properly timed spray application could be expected to control infection and to protect for a period of 5 weeks the tissues present at the time of application. Successive applications would be necessary for complete control of the disease, however, because cycloheximide would not be present in tissues formed subsequent to the application of the antibiotic.

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HOST RANGE OF A SPECIES OF TRICHODORUS AND ITS HOST-PARASITE RELATIONSHIPS ON TOMATO [1]

R. A. Rohde and W. R. Jenkins [2]

SUMMARY

In sterile soil in the greenhouse, populations of *Trichodorus* sp., the stubby-root nematode, were supported for 60 days or more by 42 different plant species representing 14 different plant families. Jimsonweed, asparagus, poinsettia, and crotalaria were the only nonhosts found. The extremely wide host range demonstrated here indicates that rotation as a means of control would be impractical in most cases.

In greenhouse-grown Chesapeake tomatoes, symptoms produced after inoculation with *Trichodorus* sp. consisted of general stunting of the entire plant plus malformation of lateral roots near the base of the stem. No distinct lesions or galls were present. Histological studies indicated that damage to roots was caused by decreased cell multiplication in root tips rather than by mechanical destruction of cells.

Feeding was observed on tomato and rye roots growing on agar plates. Under these conditions, *Trichodorus* sp. was an ectoparasite, attacking only the epidermis and possibly the outer cortex. Cell walls were punctured with the odontostylet, and the cell contents were withdrawn.

A disease of plants caused by a species of the nematode genus *Trichodorus* Cobb, 1913, was reported from Florida in 1951 by Christie and Perry (4). At that time, the name stubby root was proposed for the disease and stubby-root nematode as a name for the causal organism. The species was reported to be closely related to *T. primitivus*, but there is still some doubt as to its exact identity. Stubby root was reported to be widespread in the South and causing injury to many different kinds of plants.

Christie and Perry did not include a taxonomic description of this nematode with their original report, and subsequent authors have reported stubby root and species of *Trichodorus* from numerous locations, but there is considerable doubt that the same species was involved in all locations. According to Buhrer (1), the name stubby-root nematode may be used for any member of the genus *Trichodorus*.

Stubby-root nematodes are widespread in the southeastern United States, having been reported from Florida, Alabama, Georgia, and South Carolina (4), and also from Louisiana (10), North Carolina (14), and Maryland (16). Stubby-root symptoms have been produced experimentally on corn (4), beet (4), cowpea (9), celery (18), pea (18), and cotton (8), and population increases have been induced experimentally on soybean and sweet potato (8). Suscepts reported only from field observations are bean, cabbage, cauliflower, chayote, lima bean, pepper, sesbania, and tomato (4); peanut and onion (2); Bermuda grass and Zoysia (8); and St. Augustine grass (12). Parasitic field associations with no description of symptoms have been reported from lettuce, squash, and cucumber (15), and also from palm (3), citrus (17), avocado (5), sugarcane (13), strawberry (10), blueberry (7), and tobacco (19).

It is the purpose of this paper to report data that extend the experimental host range of 1 species of stubby-root nematodes and to describe its feeding habits and the symptoms of stubby root produced by it on tomato. The source of nematodes for inoculum was a monoculture of *Trichodorus* sp. that had been established on tomato plants in the greenhouse. Nematodes for this culture were recovered from soil around the roots of infested tomatoes in a field in Wicomico County, Maryland. Only 1 species of *Trichodorus* was present, and specimens from this population were sent to Dr. M. W. Allen, University of California, for identification of the species. This identification will be published later.

DETERMINATION OF HOST RANGE.—Plants were grown in steam-sterilized sandy-loam soil in 4-in. clay pots. Seedlings were inoculated after emergence. For small plants such as grasses or clover, several plants were left in the soil to form a dense clump, whereas larger plants such as corn and tomato were thinned to 2 or 3 plants per pot. Rooted cuttings of boxwood and azalea and runner plants of strawberry were placed singly in pots.

Two pots of each species of plant tested were inoculated with approximately 100 Trichodorus specimens each. The nematodes were transferred with a needle into a Syracuse watch glass and then pipetted into 4 or 5 holes made in the soil near the bases of the plants.

At the end of 60 days, the nematodes were removed from the soil in each pot and counted. Saprophagous forms were always present, even in sterilized soil, but no plant parasitic forms were present except Trichodorus. In cases where no stubby-root nematodes were recovered, 2 additional pots of the same plant species were inoculated in the same manner as a further test.

Control pots of soil not containing living plants also were inoculated with 100 stubby-root nematodes each to determine the survival of this species in the absence of a host. There was a gradual decline in the Trichodorus population; a few specimens were still present after 30 days, but no specimens were recovered after

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60 days. Recovery of Trichodorus specimens from soil surrounding the roots of plants after this period of time was considered as evidence that the nematodes were parasitic on the plant.

The plant species included in the experiments were arranged in 4 groups according to the number of *Trichodorus* specimens recovered 60 days after inoculation. Although the method of recovery used was not strictly quantitative, it did give a reliable indication of the relative number of nematodes present.

"Excellent" hosts were those plants upon which there was a tenfold or greater increase over the number of nematodes originally added. Plants placed in this group were as follows: GRAMINEAE—oat (*Avena sativa* L.) and tall fescue (*Festuca elatior* L.) hort. var. Kentucky 31; CRUCIFERAE — cabbage (*Brassica oleracea* var. *capitata* L.) hort. var. Late Flat Dutch, turnip (*Brassica rapa* L.) hort. var. Purple Top White Globe, and mustard (*Brassica juncea* var. *crispifolia* Bailey) hort. var. Southern Giant Curled; LEGUMINOSAE—winter vetch (*Vicia villosa* Roth), birdsfoot trefoil (*Lotus corniculatus* L.), white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), alsike clover (*Trifolium hybridum* L.), and crimson clover (*Trifolium incarnatum* L.); SOLANACEAE—tomato (*Lycopersicon esculentum* var. *commune* Bailey) hort. var. Marglobe (also Beefsteak and Chesapeake); COMPOSITAE—lettuce (*Lactuca sativa* L.) hort. var. Great Lakes and sunflower (*Helianthus annuus* L.) hort. var. Mammoth Russian.

Those plants upon which there was an increase in number, but upon which it was less than tenfold, were regarded as "good" hosts. Plants placed in this group were as follows: TAXACEAE—English yew (*Taxus baccata* L.); GRAMINEAE—field corn (*Zea mays* L.), sweet corn (*Zea mays* var. *rugosa* Bonaf.), timothy (*Phleum pratense* L.), rye grass (*Lolium perenne* L.), Kentucky bluegrass (*Poa pratensis* L.) hort. var. Merion, brome grass (*Bromus inermis* Leyss.), and Sudan grass (*Sorghum vulgare* var. *sudanense* Hitchc.); LILIACEAE—onion (*Allium cepa* L.) hort. var. Yellow Globe Danvers; POLYGONACEAE—Japanese buckwheat (*Fagopyrum esculentum* Moench.); CHENOPODIACEAE—beet (*Beta vulgaris* L.) hort. var. Detroit Dark Red; LEGUMINOSAE—soybean (*Glycine max* Merr.), alfalfa (*Medicago sativa* L.) hort. var. Williamsburg, lespedeza (*Lespedeza* sp.), and bean (*Phaseolus vulgaris* L.) hort. var. Black Valentine; ERICACEAE—azalea (*Rhododendron* sp.); CUCURBITACEAE—muskmelon (*Cucumis melo* L.) hort. var. Hearts of Gold (also Early May).

Plants were considered "poor" hosts when there was no increase in number of nematodes, but when larvae were recovered after 60 days. This was an indication that reproduction was proceeding, but at a slow rate. Plants placed in this group were as follows: GRAMINEAE—orchard grass (*Dactylis glomerata* L.) and rye (*Secale cereale* L.); CHENOPODIACEAE—spinach (*Spinacia oleracea* L.) hort. var. Bloomsdale Savoy; CRUCIFERAE—radish (*Raphanus sativus* L.) hort. var. No. 2 Scarlet Globe and rape (*Brassica napus* L.); ROSACEAE—strawberry (X *Fragaria chiloensis* var. *ananassa* Bailey) hort. var. Stelemaster (also Tennessee Shipper and Johnson A.); LEGUMINOSAE—pea (*Pisum sativum* L.) hort. var. Laxtonian; BUXACEAE—English boxwood (*Buxus sempervirens* var. *suffruticosa* L.); SOLANACEAE—tobacco (*Nicotiana tabacum* L.) hort. var. Maryland type; CUCURBITACEAE—cucumber (*Cucumis sativus* L.); COMPOSITAE—chrysanthemum (*Chrysanthemum morifolium* Ramat.).

"Nonhosts" were those plants from which stubby-root nematodes were not recovered 60 days after inoculation. Of the 46 plant species tested, only 4 were not hosts of the nematode; that is, over 90 per cent were hosts. Nonhosts were as follows: LILIACEAE—asparagus (*Asparagus officinalis* var. *altilis* L.) hort. var. Mary Washington; LEGUMINOSAE — showy crotalaria (*Crotalaria spectabilis* Roth.); EUPHORBIACEAE —poinsettia (*Euphorbia pulcherrima* Willd.); SOLANACEAE—jimsonweed (*Datura stramonium* L.).

Pathogenicity on tomato.—Tomato plants of the Chesapeake variety were inoculated in the same manner as for the host-range determinations.

When tomato seedlings on which the first foliage leaf had just developed were inoculated with 4000 Trichodorus specimens per plant, severe root injury was observed after 50 days, whereas stunting of plants was noticed after only 15 days. The 10 inoculated plants, 70 days old and inoculated 50 days previously, averaged 4.8 g fresh and 0.46 g dry weight, whereas the 10 control plants averaged 16.4 g fresh and 1.58 g dry weight (Fig. 1, A). These weights were only 29 per cent of the corresponding weights of the controls. When seedlings of the above size were inoculated with only 1000 or even with 100 nematodes each, symptoms were less severe and the effect on plant size was less than when the larger numbers were used. In some cases, lateral roots near the surface of the soil escaped injury and developed to a considerable length, but stunting of the plant was still evident.

Symptoms were always present on roots of inoculated plants, but they varied greatly in severity. The only distinctive symptoms were stunting of plants and abnormal growth of lateral roots. Lateral roots at the base of the stem were very short and showed proliferation of branch roots (Fig. 1, B). Parasitized roots were usually darker than normal in color, but there were no distinct lesions or galls such as occur in some other types of nematode injury. There was no evidence of mechanical injury of cells or of sloughing-off of tissues.

Symptoms similar to those of stubby root may result from other types of injury. Synthetic auxins, such as *m*-chlorophenyl isopropyl carbamate, may produce similar external symptoms (6). Copper residues, which accumulate in toxic concentrations after several years of application of fungicides, may also produce symptoms likely to be mistaken for stubby root. In Florida, Westgate (20) found that in certain celery seedbeds, supposedly infested with stubby-root nematodes, the plants actually were showing copper toxicity symptoms and for this reason did not respond

to fumigation.

To determine the nature of injury caused by the feeding activities of *Trichodorus* sp., root tips and small branch roots were removed from the same general areas of the root systems of inoculated and control plants. They were fixed in a formalin-aceto-alcohol mixture, dehydrated in a series of alcohols, and infiltrated and imbedded in Fisher "Tissuemat." Sections 15 μ thick were stained with safranin and fast green and mounted in Canada balsam as described by Johansen (11).

Microscopic study of sectioned root tips suggested a loss of meristematic activity by parasitized roots. There was no definite root cap or region of elongation, and the region of mitosis was much smaller in size than normal. Differentiation of cells had progressed to the extent that protoxylem elements with spiral thickenings had developed almost to the apex of the root (Fig. 1, C). In contrast to that in parasitized roots, differentiation in the control roots was initiated much further back from the root apex (Fig. 1, D). This was an indication that production of new cells had stopped in parasitized roots or at least had slowed down greatly, whereas differentiation of cells already produced continued. Branch roots were more numerous and closer together in parasitized roots than in normal roots.

OBSERVATIONS OF FEEDING.—Seeds of tomato and rye were surface sterilized with a saturated solution of calcium hypochlorite or a 3 per cent solution of hydrogen peroxide for 10 minutes and placed on the surface of a thin layer of 1.5 per cent water agar in

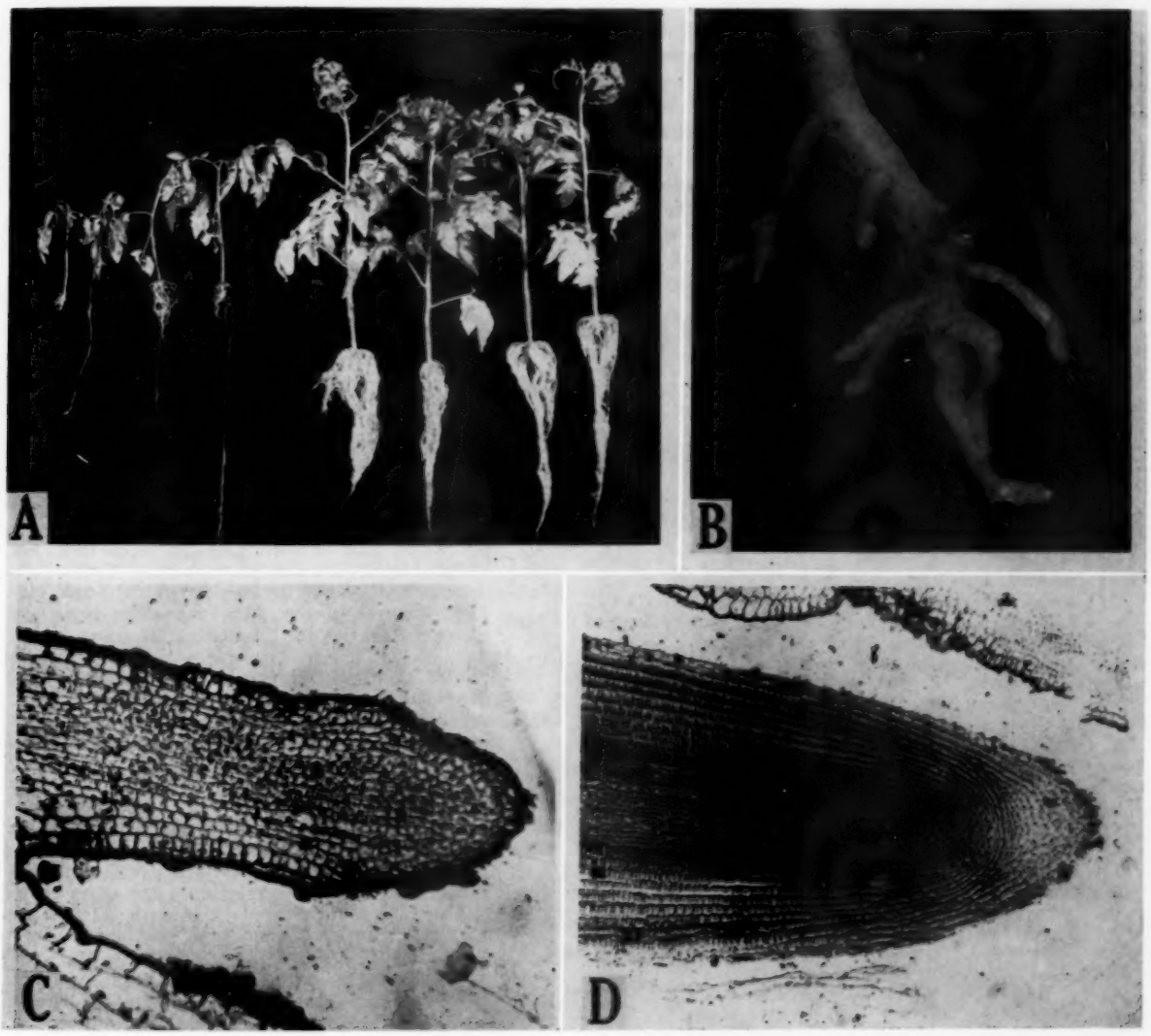

FIG. 1. Effect of a species of stubby-root nematode on tomato. A) The 4 plants on the left had been inoculated 50 days previously with 4000 nematodes each. The 4 plants on the right are checks. B) A single parasitized root showing proliferation and short branch roots. C) Photomicrograph of a section of a parasitized branch root tip. D) Photomicrograph of a section of a normal branch root tip.

sterile Petri dishes. After the seeds had germinated and root growth was initiated, stubby-root nematodes that had been rinsed several times in sterile water were washed over the surface of the roots. Bacteria and fungi introduced with the nematodes grew very slowly on the water agar and did not obscure observations. Feeding was observed under a stereomicroscope. When the Petri dish cover was removed and the site of feeding covered with a large cover glass, feeding could also be observed under a 40× oil-immersion objective of a compound microscope.

Another method used to observe feeding was to grow seedlings in a thin layer of agar held between 2 large cover glasses. Sides of this chamber were sealed with paraffin to prevent evaporation. Use of cover glasses made possible observations from both sides under the compound microscope.

Stubby-root nematodes were observed feeding directly on the roots of tomato and rye. Prior to feeding, the nematode lay on the surface of the root. The anterior portion of the nematode was then bent at a right angle, or nearly so, so that the stylet was pointed directly at an epidermal cell and was in contact with it. The stylet was rapidly thrust in and out, about 10 vibrations per second, but was never extended more than 3 or 4 μ past the lip region. Most of the motion of the stylet was in a sideways direction and was most pronounced at the base. Apparently, the cell is punctured by a rasping motion rather than by a direct thrust. At the opening of the lumen of the esophagus at the base of the buccal cavity, a convulsive "gulping" action was noticed. There was also an expansion and contraction of the basal bulb. Larvae, as well as adults, were observed feeding.

As feeding continued, the protoplast of the attacked cell shrank from the cell wall. The entire process lasted for about 5–10 seconds, and then the nematode moved on to another cell.

Feeding was restricted to epidermal cells and possibly to the outermost cells of the cortex. All areas of the root, from the root cap to the base of the stem, were attacked in the young seedlings used.

The stubby-root nematode as observed on tomato and rye is a strict ectoparasite and does not enter the root. Only living roots were parasitized, and after roots had died, the stubby-root nematodes ceased to feed.

Mechanical damage caused by Trichodorus in feeding was slight and would not account for the gross effect on roots.

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A LEAF SPOT OF PHILODENDRON RELATED TO STOMATAL EXUDATION AND TO TEMPERATURE [1]

Donald E. Munnecke and Philip A. Chandler

SUMMARY

Leaves of *Philodendron "hastatum"* growing in the Los Angeles area often have exuding spots, frequently with necrotic centers. The spots, and sooty molds growing on their exudate, which consist of from 28 to 93 per cent sugar, often make plants unsalable. Several types of bacteria were isolated from the affected areas, but attempts to inoculate plants by several techniques were unsuccessful. No control of the disease was obtained with sprays of Agri-mycin 100, copper oleate, captan, or zineb. Consequently, the disease was concluded to be a physiologic disorder. Leaf spot is severe in the warmer areas of Los Angeles but is mild in parts of the San Francisco Bay region, particularly in those cool areas close to the ocean. Also, spotting increases in the summer and decreases in the winter. No correlation was found between spotting and air pollutants or nutrition of the plant.

The disease was found experimentally to be most severe at high temperatures (75°–80°F). In 1 experiment in which plants were grown at 4 soil temperatures, the rate of growth and the rate of increase in leaf spotting were found to be exponential. The slopes of the curves showed that at soil temperatures of 60°–80° F spotting was affected more than was plant growth by a rise in temperature.

On the basis of controlled laboratory and commercial greenhouse experiments, the disease is concluded to be due to stimulation by high temperatures of exudation from secretory cells located beneath the stomates. The saprophytic bacteria associated with the spots may be responsible for the necrosis, although their role in the disorder was not demonstrated.

The disease may be partially controlled by preventing extreme temperature rises during growth and by application of fungicides to control the sooty molds that grow on the exudate.

A leaf spot of *Philodendron "hastatum"*[2] has been observed in greenhouses in the Los Angeles area for a number of years. The under surface of leaves often have exuding spots that frequently have necrotic centers. These spots, and sooty molds that grow in profusion on their exudate, often make the plants unsalable. Because of this disease, some producers in the Los Angeles area have discontinued growing the plant.

Although it has symptoms of a bacterial disease and bacteria may be found associated with the spots, the disease seems to be a physiologic disorder. It is the purpose of this paper to present data on the possible relationship of natural plant exudation, temperature, growth rate of the plant, and saprophytic bacteria to this unusual leaf spot.

SYMPTOMS OF THE DISEASE.—*Macroscopic appearance.*—The discrete spots, usually less than 2 mm in diameter, are usually randomly distributed over the lower surface of affected leaves, but they may be aligned linearly corresponding to those portions of the leaf that were in contact with the edge of the leaf sheath before unfurling. Rarely the spots coalesce to form a spot up to 5 mm in size. Usually, a colorless, sticky exudate forms in small droplets on the leaf surface upon which sooty molds subsequently develop. The spots are most numerous on old leaves (Fig. 1), sometimes nearly covering the entire surface. The first spots usually appear on leaves 2–3 weeks

FIG. 1. Leaf spot of *Philodendron "hastatum."* Note the copious exudate from the affected areas.

[1] Accepted for publication December 13, 1956.

[2] The plants referred to in commerce as *Philodendron hastatum* have no standing botanically, and Birdsey (Birdsey, M. R. 1951. The cultivated Aroids. Gillick Press, Berkeley. 139p.) states that neither of the 2 common variants he has encountered are *Philodendron hastatum* Koch & Sellow. Since there is no other name in use for these plants, we have adopted Birdsey's use of quotation marks with the specific name.

after they unfurl, although they have been observed within a week after unfurling. No necrosis is associated with early spotting, but as the spots age they frequently become necrotic. Only the necrotic spots are visible on the upper leaf surface.

Water soaking, sometimes seen along the base of the midrib of very young leaves, disappears as the leaf matures; this condition apparently has no relation to leaf spotting.

The exudate is watery on young leaves growing in a moist atmosphere, but may become thick like syrup on old moribund leaves. The percentage sugar content (as determined by an Abbe refractometer) of the exudate on old spots on senescent leaves was found to be 93 per cent, whereas that of exudates on young spots on immature leaves was 28 per cent. The high sugar content of old spots is due to concentration by evaporation of the liquid phase. The exudate contained a reducing sugar as determined by Benedict's solution.

The fact that stomates of *P.* "*hastatum*" may guttate readily was determined experimentally. Water was withheld for several days from a plant to create a water stress. Then an air current was blown over the leaves for several hours, and the plant was enclosed in a bell jar overnight in a cool greenhouse (65°F). Next morning, the lower surface of the leaves was covered with droplets that had exuded from the stomates. The ability of plants to guttate through stomates is not uncommon,[3] but the high sugar content of the guttate of *P.* "*hastatum*" appears to be unusual.

Microscopic appearance.—Examinations of freehand sections of the leaf spots disclosed large numbers of bacteria between the cells around the stomates but very few in the fresh exudate. Bacteria were especially numerous in necrotic cells, but none were found inside healthy cells. The spots, centered on the stomates, were confined to the lower surface of the leaf. Cells beneath some of the stomates were larger and more specialized than the normal mesophyll cells and appeared to produce the guttation liquid. Muller[4] studied the anatomy of the hydathodes of Araceae and reported that stomates of *Philodendron* spp. frequently have a secretory function. In one type, he found that tracheids that end near the stomates or hydathodes secrete sap into the substomatal cavity. In another type, sap was secreted from 1 or more cell layers beneath the stomates into the substomatal cavity. Apparently the stomates of *P.* "*hastatum*" are of the second type described by Muller.

Field observations of the disease.—The disorder is severe in the Los Angeles area but is practically unknown in parts of the San Francisco Bay region, particularly in those areas close to the ocean. It appears that, in California, leaf spotting is least prevalent where temperatures are cool and equable.

Isolations.—Attempts to isolate pathogenic organisms from affected leaf areas were unsuccessful. Numerous isolations were made to potato-dextrose agar, beef-peptone agar, beef-peptone broth, and sucrose agar. No predominant bacterial type was isolated consistently nor were fungus pathogens obtained. The bacteria most frequently isolated formed white or yellow colonies on potato-dextrose agar and were small gram-negative rods.

Inoculations.—Over a period of 2 years, plants of *P.* "*hastatum*" were inoculated with the various bacteria isolated from the leaf spots. The inoculum consisted of single isolates or various combinations of the isolates. Leaves of plants were inoculated with bacterial suspensions by spraying, by hypodermic injection, or by vacuum impregnation. Since it was impossible to obtain plants free of leaf spots, the results were evaluated by noting whether there was a significant increase in the number of spots per leaf on the inoculated plants as compared to similarly treated noninoculated plants. None of the bacteria was found capable of infecting the plant and causing the typical leaf spot. It was therefore concluded that the disease was primarily physiologic in nature.

Relation of air pollutants or nutrition.—In the Los Angeles basin, air pollutants conceivably could be a contributing factor to the formation of spots, although this symptom is not one of the known responses of plants to air pollutants.[5] Subjecting a series of plants to various dosages of artifically produced recognized air pollutants did not increase spotting or exudation, nor did it have any visible detrimental effect on the growth of the plants. Also, in the 5 years that the problem has been studied, there has been no observed correlation between the incidence of leaf spotting and the presence or absence of air pollutants.

The disease does not seem to be due to a nutrient deficiency or excess because it occurs where different soil mixes are used, and various fertilizer schedules have no effect on it.

Effect of conventional control treatments on leaf spotting.—Various conventional control treatments with fungicides and bactericides were tried. Twenty-four plants, sorted as to size, number of spots per leaf, and number of leaves, were divided into 4 lots. The old leaves that were heavily spotted or moribund were removed. The spots on each remaining leaf were counted and recorded. The treatments were Bordeaux mixture (2.5–2.5–50), spray thrice weekly; Greenol (15 per cent copper oleate), 1–800 dilution of formulated product, thrice weekly; water swab, thrice weekly (a cotton ball dipped in a small quantity of distilled water was used to wash both surfaces of the leaves of all plants); and control (leaves kept dry during the experiment). The experi-

[3] Bald, J. G. 1952. Stomatal droplets and the penetration of leaves by plant pathogens. Amer. Jour. Bot. 39: 97-99.

[4] Muller, Lene. 1919. Hydathoden der Araceae. Akad. der Wiss. Wien, Math.-Nat. Kl. Sitzber. 128: 665-694.

[5] Middleton, J. T., A. S. Crafts, R. F. Brewer, and O. C. Taylor. 1956. Plant damage by air pollution. Calif. Agr. 10 (6): 9-12.

ment was continued for 6 weeks. The effectiveness of the treatments was evaluated by counting the spots per leaf at weekly intervals.

The most striking result was the control obtained by washing the leaves with water (Table 1). If the bacteria were actively parasitic, the deliberate spreading of them in the wash water would tend to increase, rather than decrease, the incidence of spotting. This lent credence to the other data, which indicated that the bacteria associated with the leaf spotting were not the primary causal agents. In several other experiments, Agri-mycin 100 (15 per cent streptomycin and 1.5 per cent oxytetracycline), 100 ppm of active ingredients, and 2 fungicides, captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide) and zineb (zinc ethylenebis[dithiocarbamate]), 1½ lb. of 50 per cent and 65 per cent formulated products respectively, per 100 gal. were sprayed on the plants at weekly intervals. They did not appreciably reduce the incidence of spotting.

Several attempts were made to control leaf spotting in a commercial greenhouse. In one attempt, plants were syringed 4 times daily for 3 months, but the incidence of leaf spotting was unaffected by the treatment. In another trial, plants in ground beds from which cuttings were continuously harvested were divided into blocks of approximately 100 sq. ft. each. Half of each block was sprayed weekly and the other half sprayed every other week with the following: zineb, 1½ lb. of the formulated material per 100 gal. of water; captan, 3 lb. of the formulated material per 100 gal. of water; Agri-mycin 100, 100 ppm active ingredient; and Greenol, 1:500, formulated product. Controls were given no treatment. A record of the number of spots per leaf was made at the time the cuttings were taken and again after the cuttings were rooted. The Greenol and Agri-mycin 100 treatments were discontinued after 6 months, but the other treatments were continued for 14 months. None of these treatments controlled leaf spot, but adequate coverage with captan or with zineb every other week effectively controlled the sooty molds. Actually, the control of sooty molds is more important economically than is the control of leaf spot, since the black discoloration of the plants is the primary reason for unsalable plants.

An attempt was made to control the disease by treating 8-in. stem sections with hot water and then growing the plants in steamed soil. The succulent cane taken from the top of the plant was treated separately from the less succulent cane taken from the lower portions of the plant. All sections survived immersion in water held at 120°F for 30 minutes. Immersion in water at 124°F for 30 minutes killed all sections from the top of the plant and most of those from bottom portions. The plants grown from surviving pieces were observed for a year, but no control of leaf spot was obtained.

TABLE 1.—*Incidence of leaf spot on plants of Philodendron "hastatum" sprayed thrice weekly with fungicides or washed thrice weekly with water*

Treatment	Mean no. of spots per leaf		Percentage increase
	Initial	Final	
None	14	36	260
Bordeaux mixture (2.5-2.5-50)	8	15	187
Greenol (1:800)	8.5	15.5	180
Water swabbing	13.5	18.1	130

ENVIRONMENTAL RELATIONS.—*Effect of air temperature on leaf spotting.*—One lot of 6 plants was grown in the greenhouse at an average temperature of 63°F (cool house). Another lot was grown at an average temperature of 70°F (warm house). A third lot was grown in the cool house during the day and transferred to the warm house at night. A fourth lot was grown in the warm house during the day and moved into the cool house at night. The mean increase in the number of spots per leaf for the 6-week duration of the experiment was as follows: continuously warm, 29; warm days and cool nights, 21; cool days and warm nights, 21; and continuously cool, 7. From these results, it was apparent that most spotting developed when plants were grown at the higher temperatures. Also, it appeared that the day/night temperature relationship was not critical.

Although the rate of growth was not measured, the plants did not grow nearly as rapidly in the cool house as they did in the warm house. This may have affected the incidence of leaf spot.

In another experiment, 1 greenhouse chamber was maintained at a minimum temperature of 75°F and a maximum of 85°–90°F. In another chamber, the temperatures fluctuated from a minimum of 75°F to a maximum of from 90°–105°F, depending upon the heat of the day. In each chamber, 1 lot of plants was syringed daily by squirting water from a hose on both leaf surfaces. The leaves of the other lot were kept dry.

The comparative rate of growth of the plants was determined by counting the number of leaves at the beginning and at the end of the experiment. Since the leaves make up most of the structure of *P. "hastatum,"* a leaf count is an accurate estimate of the rate of growth. The incidence of spotting was determined by making counts of the number of spots per leaf at the beginning of the experiment and monthly counts thereafter.

The results from this experiment were similar to those of the previous one. That is, the plants grew faster and leaf spotting was more prevalent at the higher temperatures than at the lower ones. The percentage increase in the number of leaves produced in the uncontrolled environment divided by the percentage increase in the number of leaves produced in the controlled environment was 1.5. The increase of the mean number of spots per leaf in the uncontrolled environment divided by the increase of the mean number of spots per leaf in the controlled environment also was 1.5. The fact that the 2 ratios were identical does not necessarily indicate a corre-

lation of rate of growth with incidence of leaf spot; however, it is another indication of the interrelationship of temperature, growth rate, and incidence of spotting.

Syringing had no effect upon the rate of growth of the plants, but it appeared to reduce the incidence of spotting in the uncontrolled temperature series.

Data taken in the commercial greenhouse mentioned previously indicated that the incidence of spotting increased during spring to a maximum in late summer, then decreased to a minimum in the winter, and again increased rapidly the following summer. Again, the periods of greatest incidence of spotting were correlated with the periods of highest temperature.

Effect of soil temperatures on leaf spot.—The effect of soil temperatures on the rate of growth and leaf spot was determined by growing lots of 5 plants each in temperature tanks held at 50°, 60°, 70°, and 80°F. Newly rooted cuttings having an average of 2 leaves per plant were used for the experiment, which lasted for 4 months.

The plants appeared to be dying at the 50° temperature, and several of the leaves dropped off soon after unfurling. Plants grown at 60° were greener and more vigorous than those grown at the lower temperature, but there was considerable chlorosis. The optimum temperature for growth, judging by the green color of the leaves and the apparent vigor of the plant, was near 70°, although a few more leaves were produced at soil temperatures of 80°. Plants grown at the highest temperature were slightly yellow and not as vigorous as those grown at 70°F.

Analysis of the data showed a very significant relationship of soil temperature to leaf spotting and growth of the plant. The data were taken monthly by counting the number of spots per leaf and the number of leaves per plant. The logarithm of the increase in mean number of spots per leaf and the increase in total number of leaves produced at each soil temperature for the 4 month period of the experiment are plotted on Figure 2. The curves show that spotting and plant growth increased linearly from 60° to 80°F. The divergence of the curves at 50°F is probably due to the extremely abnormal growth of the plants at that temperature. The slopes of the 2 curves show that soil temperature affects leaf spotting more than it affects plant growth. This was the first conclusive experimental evidence which showed this relationship.

Discussion.—This leaf spot of *Philodendron "hastatum"* presents many interesting relationships of growth, stomatal exudation, and temperature. As is the case with many physiologic disorders of plants, it is difficult to separate the effect of one of the environmental interactants (e.g. temperature) from other factors. Thus, *P. "hastatum"* grows more rapidly at high temperatures than at lower ones and leaf spotting increases at the high temperatures. It was possible to separate these effects by controlling soil temperatures, and it appears conclusive that high temperatures increase spotting directly, apart from the effect on the plant growth. The experimental data agree closely with observations made in commercial greenhouses.

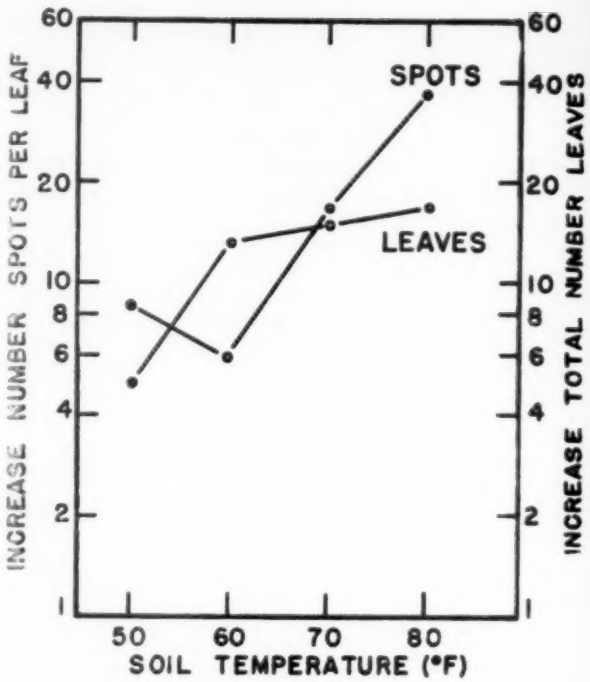

Fig. 2. Effect of soil temperatures on the incidence of leaf spot and the increase in total number of leaves of *Philodendron "hastatum."*

In many respects, the leaf spot of *P. "hastatum"* has symptoms characteristic of a bacterial leaf spot disease; however, the bacteria found in the necrotic areas of the spots appear to be nonpathogenic, and various bactericides were ineffective in checking the disease. In fact, the best control was obtained by washing the leaves with a cotton swab in such a way that the bacteria were deliberately spread over both leaf surfaces rather than concentrated around the stomates. Probably the disease could be controlled by washing the leaves in this way, but it is uneconomical commercially. Attempts to extend the method by syringing were unsuccessful, since it is impossible to wet the lower leaf surfaces adequately because of the dense foliage and overlapping leaves on plants grown in commercial ranges.

Although the role of bacteria in the production of the necrosis was not proved, the evidence favors the postulate that the leaf spot of *P. "hastatum"* is due to plant exudation stimulated by high temperatures and rapid growth of the plant and that the necrosis frequently associated with the spotting may be due to the action of saprophytic bacteria whose products injure the adjacent cells.

The combination of physiologic and environmental factors makes the control of leaf spot very difficult when plants are grown at high temperatures. The

plants grow rapidly at the high temperatures, and this decreases the cost of production tremendously. The problem of maintaining maximum production of plants with a minimum of spotting is a difficult one to solve. One partial solution has been to prevent extreme temperature rises during growth and to control by applications of fungicides the sooty molds that grow on the exudate. In this way, the grower may minimize the effects of the disorder and continue to produce high quality plants.

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PECTOLYTIC ENZYMES PRODUCED BY SCLEROTINIA SCLEROTIORUM[1]

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SUMMARY

Sclerotinia sclerotiorum (Lib.) d By. produced pectin methyl esterase and polygalacturonase on wheat bran. The optimum pH for enzyme activity of the culture filtrate was pH 4 when determined by rate of maceration of carrot root tissue and by reduction in viscosity of sodium polypectate solution. The activity was destroyed on exposure for 10 minutes to a temperature of 55°C. The extracts of both susceptible (radish, cucumber, and carrot) and resistant (potato and onion) vegetable storage organs reduced the maceration activity of the filtrate; onion extract completely inhibited activity. The pectolytic enzymes of *S. sclerotiorum* differ from that previously described for the bacterial soft-rot organism, *Erwinia aroideae* (Towns.) Holland, in that the latter produced pectin depolymerase but little or no pectin methyl esterase and no polygalacturonase. The depolymerase of *E. aroideae* was most active at pH 9, and activity was not destroyed completely by exposure to a temperature of 65°.

Sclerotinia sclerotiorum (Lib.) d By. incites one of the common soft rots of vegetables. The disease, as it occurs on fleshy roots, fruits, and other storage organs, has for a long time been referred to in market pathology in the United States as "watery soft rot." Rotting of the same organs by one or another of the soft-rotting species of *Erwinia* is, by contrast, referred to as "slimy soft rot." The descriptive adjectives refer to the consistency of the rotting substrate. As implied, the substrate parasitized by *S. sclerotiorum* tends to release watery liquid much more readily than does that parasitized by Erwinia. The enzyme produced by *E. aroideae* (Towns.) Holland was found to be pectin depolymerase, which is capable of breaking the pectin chain into polygalacturonic units but incapable of hydrolysing these units into monogalacturonic acid (9). The present investigation was directed toward a study of the nature of pectolytic enzymes produced by *S. sclerotiorum*.

Experimental results.—The culture of *S. sclerotiorum* used was furnished by N. N. Winstead, North Carolina State College. It conformed in cultural characteristics with descriptions of the large-sclerotium form of the species as described by Purdy (4). The fungus was grown in 32-oz. pharmaceutical bottles, each containing 25 g of moist wheat bran. After 10 days at 22°C, 200 ml of distilled water and 2 drops of toluene were added to each bottle, and autolysis was allowed to proceed for 12 hours. The extract was filtered through cheesecloth, and the filtrate was centrifuged. The clear supernatant was stored at 4° for future use.

Determination of the enzymes.—Ten-ml portions of 0.5M acetate buffer (pH 4.5) were added to 75-ml portions of 1.5 per cent commercial pectin (about 9 per cent methylated) in 250-ml beakers. The wheat-bran culture filtrate was adjusted to pH 7, and assay samples of 5, 10, and 15 ml (brought up to 15 ml in each case with distilled water to keep the volume constant) were added, with constant stirring, to the pectin-buffer mixture. Checks were run with 5, 10, and 15 ml of heated filtrate. After 3 hours at 31°C, the solutions were adjusted to pH 7 by addition, with constant stirring, of 0.1 N alkali. The difference between the number of ml of 0.1 N alkali required for the sample and that required by the check equalled the ml of 0.1 N acid released by enzyme activity. This amount multiplied by 3.1 gave the mg of methoxyl removed by the enzyme sample in 3 hours.

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A correction for the dilution factor and size of the sample was made; this yielded the pectin methyl esterase (PME) activity in mg methoxyl removed per ml of culture filtrate. After 3 hours, an average of 4.09 mg methoxyl was removed per ml. This showed a high PME activity for *Sclerotinia sclerotiorum* when compared with *Botrytis cinerea* Fr. (1.912 mg) and with several forms of *Fusarium oxysporum* Schlect. (0.207-0.547 mg) grown on the same medium as reported by Winstead and Walker (8).

In order to study the hydrolytic action of the enzymes involved, 1.5 per cent commercial pectin (about 9 per cent methylated) or 0.5 per cent sodium polypectate solutions was added to portions of the wheat-bran culture filtrate. The reducing groups formed were assayed by the method described by Winstead and Walker (8). The results obtained were expressed in ml of 0.1 N iodine solution required to reduce 10 ml of reaction mixture. The results of a typical experiment (Fig. 1, A) show increasing production of reducing groups over a period of 3 hours, with very similar reactions on pectate and pectin substrate. The filtrate was tested also for reduction in viscosity of pectin and pectate solutions. Five-ml samples were placed in an Oswald viscosimeter, and the time of flow was recorded. The results of a typical experiment are shown in Figure 1, B. Again the enzymes were about equally active on pectin and pectate; on both substrates, about 90 per cent reduction in viscosity was attained in 15 minutes.

Since a strong hydrolytic activity was indicated, the next step was to determine whether the enzyme concerned was a depolymerase (DP) or a polygalacturonase (PG). The latter would be indicated by presence of monogalacturonic acid in the reaction mixture. A mixture of 10 ml of the filtrate and 25 ml of 0.5 per cent sodium polypectate was prepared, and samples were removed at 24 and 48 hours. The samples were analyzed by ascending paper chromatography in which Whatman No. 1 filter paper was used with a solvent system containing 1-butanol, acetic acid, and water (4:1:5). A solution of monogalacturonic acid was used as a control. When the solutions had risen to about 1 in. from the top, the paper was removed, dried, and sprayed with a solution made up of 0.5 g benzedine, 10 ml acetic acid, 10 ml 45 per cent trichloroacetic acid, and 100 ml 95 per cent ethanol. It was then placed in an oven at 90°C for 2 minutes and then examined. Monogalacturonic acid in the control was indicated by a pink spot. The filtrate with pectin and that with pectate produced pronounced pink spots at the same level as did the control at 24 and at 48 hours, showing conclusively that PG was present in the enzyme mixture. It is also probable that DP was present, but there is no feasible method of determining the presence or extent of the latter when PG is also contained in the enzyme complex.

Relation of pH and heat to enzyme activity.—Since the pH optimum for hydrolytic activity of pectolytic enzymes varies among enzymes produced by different organisms (2), it was of interest to determine the optimum for the Sclerotinia enzyme mixture. This was done in 2 ways. Blocks of carrot root 1 cm square were cut on a microtome into slices 200 μ thick. They were sterilized in a dilute solution of sodium hypochlorite, then immersed in 5-ml portions of wheat-bran filtrate to which 2 ml of McIlvaine's buffer solution had been added. By means of the buffer solution, the pH of the various samples was adjusted over a range of pH 3 to pH 7. The activity of the enzyme was determined as the number of minutes required for maceration to proceed to the point at which the slice could not be lifted by the edge with a forceps. The results of a typical experiment are presented in Table 1. Most rapid maceration occurred at pH 4.

The second method of determining the optimum pH for hydrolytic activity was by determination of reducing groups formed by samples of the filtrate to which 0.5 per cent sodium polypectate solution was added. The mixtures were adjusted to a series of pH levels. The results were expressed as ml of 0.1 N iodine solution reduced by 10 ml of the reaction mixture. The data from a typical experiment are presented in Figure 1, C. The greatest activity also occurred at pH 4 when determined by this method.

Overell (3) reported that oxalic acid was produced in old cultures of *S. sclerotiorum*. The filtrate from a 10-day wheat-bran culture was assayed for oxalic acid by the chromatographic method of Scott (7). Oxalic acid was shown to be present in the filtrate; however, when a saturated solution of calcium chloride was added to the filtrate, no calcium oxalate was precipitated, indicating that the oxalic acid present was less than 0.0005 M.

Five-ml portions of wheat-bran filtrate were heated in thin-walled test tubes for 10 minutes at various temperatures from 30° to 100°C. Enzyme activity was

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FIG. 1. Pectolytic enzyme activity in the filtrate of a wheat-bran culture of *Sclerotinia sclerotiorum*. A) Relative rate of hydrolysis of sodium polypectate and of pectin by enzymes in the filtrate, as determined by measurement of the reducing groups produced. B) Relative rate of reduction in viscosity of the same substrates as in A. C) The relation of pH of the filtrate to the hydrolytic activity of the enzymes, as determined by measurement of the reducing groups produced. D) The pectolytic activity of the filtrate after exposure to different temperatures for a 10-minute period as determined by measurement of the reducing groups produced.

determined by assaying reducing groups produced in a 0.5 per cent sodium polypectate solution. There was a progressive decline in activity, with complete inactivation at 55° (Fig. 1, D).

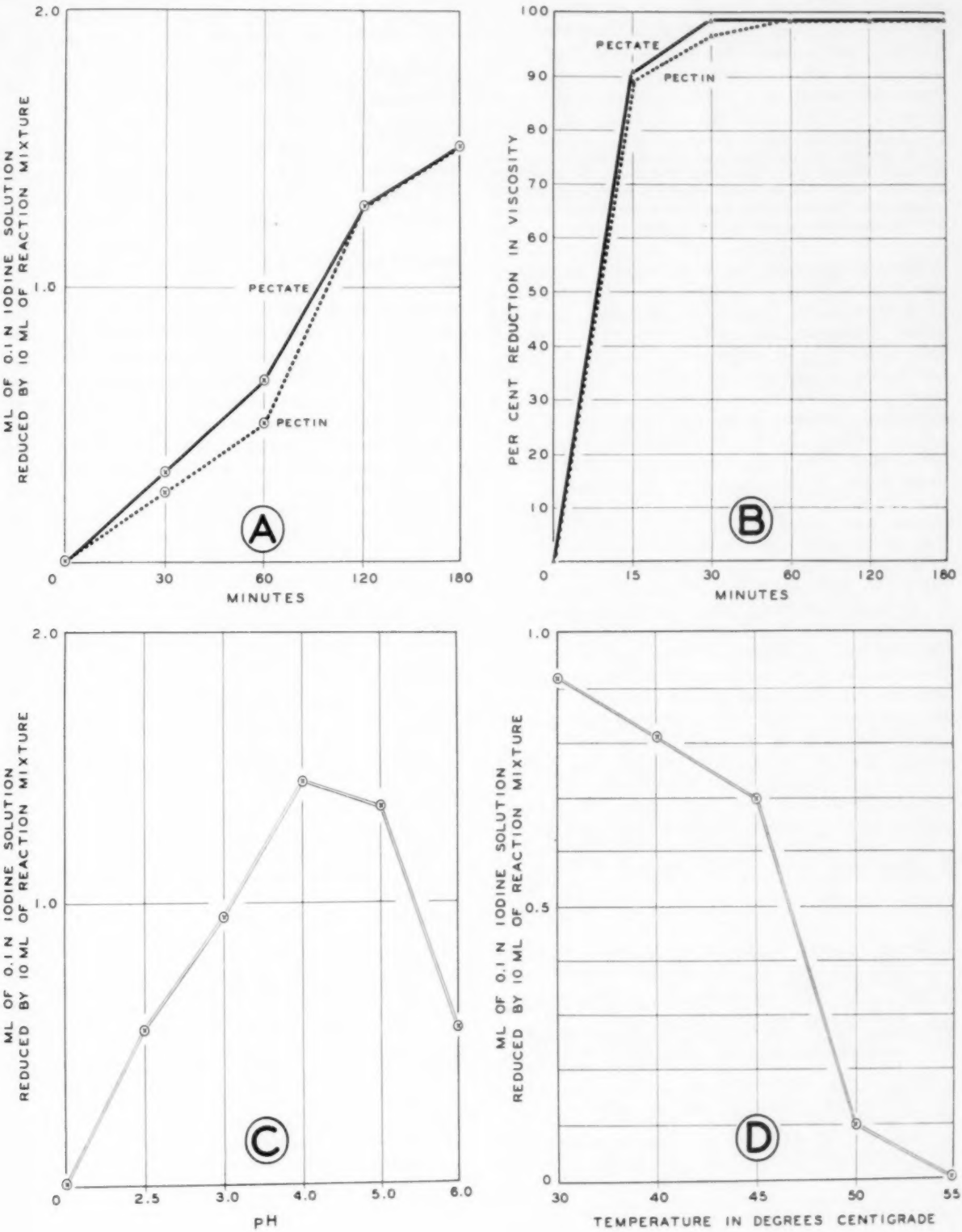

TABLE 1.—*Maceration of carrot root slices in filtrate from a wheat-bran culture of Sclerotinia sclerotiorum adjusted to various pH levels*

pH	Maceration time (minutes)	Relative activity[a]
3	25	80
4	20	100
5	35	57
6	45	44
7	80	20

[a] A relative activity value of 100 was assigned to the adjusted filtrate that macerated the slices most rapidly. For each other test, the time required for the most rapid sample was divided by the time of the sample in question, and the quotient was multiplied by 100.

TABLE 2.—*Maceration activity of filtrate from wheat-bran culture of Sclerotinia sclerotiorum on carrot blocks in the presence of juices of various vegetables*

Source of juice	pH of juice	Time (minutes) required for complete maceration in: Filtrate plus natural juice	Filtrate plus juice adjusted to pH 4
Radish root	6.6	105	70
Cucumber fruit	5.8	125	100
Carrot root	6.6	135	110
Potato tuber	6.3	145	140
Onion bulb	5.4	—[a]	—[a]
Water control	6.5	80	70

[a] No maceration after 900 minutes.

Effect of vegetable juices on enzyme activity.—*S. sclerotiorum* incites a rapid soft rot of storage organs of a considerable number of vegetables, including carrot, cucumber, and radish; however, it induces little or no decay of potato tuber tissue (5, 6) and is not known to parasitize onion bulbs. A study was made of the effects of juice extracted from the above-mentioned vegetables on the macerating activity of the culture filtrate. Juices were expressed from radish roots, carrot roots, cucumber fruits, potato tubers, and onion bulbs. These juices were centrifuged at 3000 rpm for 10 minutes, and 2-ml portions of the supernatants were added to 5-ml samples of the filtrate. Two ml of water was substituted for vegetable juice as a control. Half of the samples were adjusted to pH 4 with McIlvaine's buffer solution. Carrot blocks 1 cm square and 200 μ thick were inserted, and the time required for complete maceration was recorded. The activities in unadjusted juice and in juice adjusted to pH 4, the optimum for maceration activity, were less than that in the control (Table 2). Maceration was faster in each case in adjusted than in unadjusted juice. It is of interest that activity was greater in juice from the 3 susceptible hosts than in that of the 2 resistant hosts, although the differences in maceration time were not large. The juice of onion, however, completely inhibited pectolytic activity.

DISCUSSION.—The pectolytic enzymes produced by *S. sclerotiorum* on wheat bran consisted of both pectin methyl esterase (PME) and polygalacturonase (PG). This soft-rotting pathogen differs, therefore, from *E. aroideae*, which produces depolymerase (DP) and little or no PME. The production of PME may account for the fact that partially methylated pectin was hydrolyzed as rapidly as was sodium polypectate. The maceration activity on carrot and the reduction of viscosity of pectate solution were greatest at pH 4, whereas the activity of the enzyme of *E. aroideae* was greatest at pH 9. The activity of the Sclerotinia enzymes was destroyed by exposure to 55°C for 10 minutes, whereas that of the Erwinia enzyme was not destroyed completely at 65° (9). The differences between the types of rot induced by Sclerotinia and by Erwinia probably are in part due to the different types of enzymes produced by the 2 organisms. Another difference between the 2 organisms that undoubtedly affects the type of rot is that Sclerotinia mycelium penetrates cell walls (1), whereas the soft-rot bacteria do not ordinarily invade the host-cell lumen. The fact that onion bulb extract is completely inhibitive to the Sclerotinia enzyme may be 1 basis for the resistance of that species, but further study is necessary before a conclusive statement on this point could be made.

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PHYTOPATHOLOGICAL NOTES

A Modification of Bald's Stain for Viruses and for Cell Inclusions Associated with Virus Infections. T. E. RAWLINS. The purple color produced by Bald's[1] stain when applied to pure virus or to cell inclusions associated with virus infections is apparently the most specific biological stain for plant viruses that has been described. Since the original method has given somewhat variable results for us, we have tried modifications of the method to obtain more reproducible results.

The following modification of Bald's schedule was found to give consistent results. 1) Peel epidermal strips from tobacco leaves infected with tobacco mosaic virus, float on tap water, cut with a sharp razor blade into pieces suitable for mounting, and transfer to the fixing solution by means of a glass rod. 2) Fix in Bald's alcohol-iodine-formalin fixing solution for at least 80 minutes.[2] 3) Wash strips in 0.25 per cent sodium thiosulfate in 50 per cent ethyl alcohol for 10 minutes to remove the iodine. 4) Wash strips in 50 per cent ethyl alcohol for 10 minutes, in 70 per cent ethyl alcohol for 10 minutes, and in 95 per cent ethyl alcohol for 10 minutes or longer. 5) Pour the epidermal strips into a large dish of water and leave 15 minutes. Add drops of distilled water to wet the upper surface of the strips. 6) Pick up a strip on a microscope slide. 7) Absorb the water around the strip on filter paper. 8) Cover the strip with several drops of undiluted Giemsa stain (without orange G) for about 7 minutes. (Harleco Giemsa blood stain, stock solution, made by Hartman-Leddon Co., Philadelphia, was used.) 9) Wash the strip in several changes of distilled water in a 400-ml beaker for 20 minutes. The strip will appear purple after this treatment. 10) Pour the strip into a large dish of water and again pick it up on a microscope slide. 11) Absorb the water from around the strip on filter paper. 12) Add several drops of absolute ethyl alcohol to the strip and leave for only several seconds. Place white paper under the disposal beaker, so that the color change in the strip may be seen. As soon as the strip changes from a purple color to a blue or green color, quickly pour off the alcohol and add several drops of clove oil to the strip. If left in the alcohol too long, the cell inclusions will become blue to green, like the host chromatin and cell walls. 13) After 1 or more minutes, pour off the clove oil, replace with fresh clove oil, and continue to add and pour off clove oil until the discarded oil shows no green color and the cell inclusions are well differentiated when viewed under the low power of the microscope. It may require 10 minutes or more to obtain the best differentiation. 14) Pour off the clove oil, and absorb the remainder from around the strip on filter paper. 15) Flood the slide with xylene to remove the clove oil and continue to change the xylene at intervals for 10 minutes. Gently move the strip on the slide during this treatment so that the xylene will wash the clove oil from both surfaces of the strip. Do not allow the strip to become dry. 16) Pour off the xylene, absorb that around the strip, and mount in a 60 per cent solution of H-S-R Dried Resin Microscopic Mounting Medium in toluene or xylene. This resin is sold by Hartman-Leddon Co., Philadelphia, Pa.

The crystal-like cell inclusions associated with tobacco mosaic virus infection have been shown by Steere and Williams[3] and Rubio[4] to be composed mainly or entirely of rod-shaped virus particles. These inclusions stain purple, as do the vacuolate X-bodies and sometimes the nucleoli. The nucleolus, like the virus, is rich in ribonucleic acid. The host chromatin and cell walls stain green to blue. The method is not very satisfactory for cells that contain chloroplasts, since the chloroplasts may also stain purple. It is supposed that there is sufficient ribonucleic acid in the chloroplasts to account for this.

Neither this method nor the Sakaguchi histochemical method[5] for viruses is completely specific for plant viruses; however, in our experience each method is sufficiently specific to have considerable value in histological studies of virus-infected tissues.—Department of Plant Pathology, University of California, Berkeley 4, California.

Mosaic and Shoestring, Virus Diseases of Cultivated Blueberry in New Jersey[1]. E. H. VARNEY[2]. Mosaic and shoestring have been observed for a number of years in various blueberry plantings in New Jersey. Shoestring was generally assumed to be a virus disease, but mosaic, referred to locally as "variegation," was thought to be a genetic disorder. This paper describes the symptoms of the 2 diseases and gives evidence based on limited transmission experiments

[1] Bald, J. G. 1949. A method for the selective staining of viruses in infected plant tissues. Phytopathology 39: 395-402.

[2] The fixing solution, suggested by Dr. Bald, had the following composition: 2 ml of solution containing 4 g iodine and 6 g KI in 100 ml water; 5 ml absolute ethyl alcohol; 2 ml distilled water; and 1 ml formalin. The formalin should not be added until just before use.

[3] Steere, R. L., and R. C. Williams. 1953. Identification of crystalline inclusion bodies extracted intact from plant cells infected with tobacco mosaic virus. Am. J. Botany 40:81-84.

[4] Rubio, M. 1954. Rapid extraction of intact crystalline inclusions from the cells of plants infected with tobacco mosaic virus. Nature 174:313.

[5] Rawlins, T. E., A. J. Weierich, and D. E. Schlegel. 1956. A histochemical study of certain plant viruses by means of the Sakaguchi reaction for arginine. Virology 2:308-311.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, New Brunswick.

[2] Formerly, Plant Pathologist, Horticultural Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

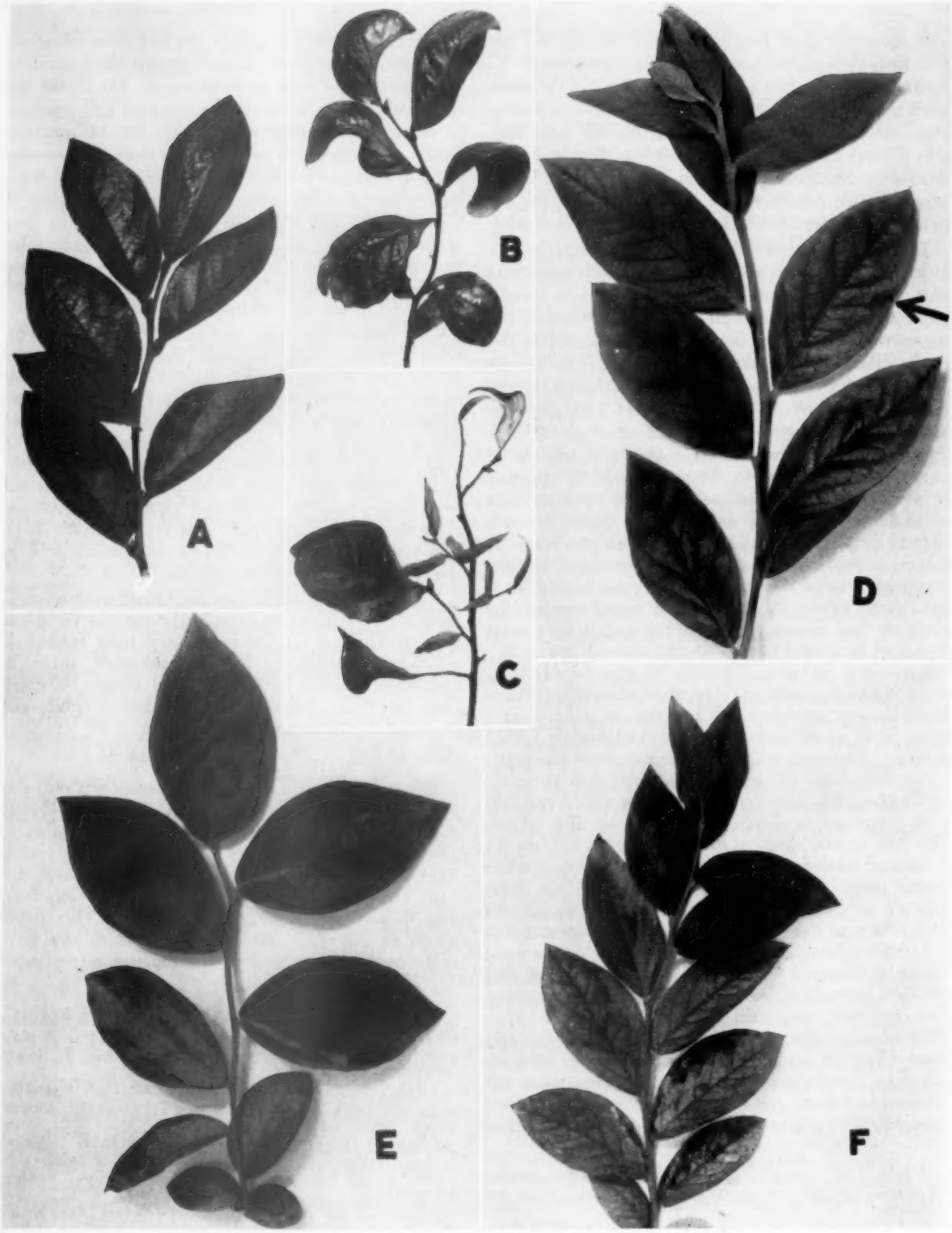

FIG. 1. A) Normal twig of Burlington blueberry; B and C) Naturally infected Burlington twigs showing leaf distortion caused by shoestring virus; D) Naturally infected Cabot twig showing red streaks on stem and red veinbanding (arrow) caused by shoestring virus; E) Stanley and F) Cabot blueberries artificially inoculated with mosaic virus.

that both are of virus origin.

Blueberry plants infected with shoestring virus show both twig and leaf symptoms. Red streaks of varying length occur in the spring on new twigs, especially the surface exposed to direct light (Fig. 1, D). As the summer progresses, the streaks become progressively less evident and are finally masked as the wood matures. Severely affected leaves are narrow and pointed or straplike (Fig. 1, C). Moderately affected leaves are wavy and distorted as a result of the abnormal development of the leaf tissue (Fig. 1, B). Affected leaves may be green or dull red. Red veinbanding, a common symptom on both normally shaped and malformed leaves, may be limited to a portion of a vein (Fig. 1, D) or may be so extensive that most of the leaf is red. Veinbanding is often limited to the midvein and to the bases of the lateral veins, resulting in a conspicuous oak-leaf type pattern. Diseased twigs may be scattered among apparently normal branches, and a symptomless twig may occur on a severely diseased branch. Blueberry seedlings and water shoots of named varieties occasionally are affected by a disorder that superficially resembles shoestring. Leaves are narrow, usually rugose, and frequently marked with areas lacking chlorophyll. This disorder has not been proved to be caused by an infectious agent.

Leaves of mosaic-affected blueberry are strikingly mottled with yellow and yellow-green areas (Fig. 1, E, F). Some leaves are yellow only along margins of the major veins. The degree of mottling varies with leaf position and blueberry variety. Lower leaves on a twig (Fig. 1, F) may be predominantly yellow, whereas the upper leaves may show no symptoms. Mottle on leaves of the Cabot variety is conspicuous at first, but frequently fades as summer progresses. In contrast, mottle on leaves of the Stanley variety becomes more brilliant and colorful as yellow areas turn various shades of red and orange-red. Observations suggest that a bush with 1 or 2 affected branches was recently infected. Usually all branches show symptoms in another 2 to 3 years, depending upon the size of the bush. Genetic variegations that resemble the virus-induced mosaic occur frequently among blueberry seedlings.

During the winter of 1954, 10 Stanley and 9 Cabot blueberry plants were inoculated at budbreak in the greenhouse by means of chipbuds from a Stanley bush showing symptoms typical of mosaic. A few leaves on all inoculated plants showed typical symptoms 2 to 3 weeks after inoculation. All branches of the inoculated plants were affected the following year (Fig. 1, E, F).

Two Jersey and 2 Burlington plants were inoculated in the spring of 1953 by means of chipbuds from a Cabot plant showing typical shoestring symptoms. These plants had leafed out at the time of inoculation, and no symptoms developed the first year. The following spring, inoculated plants showed the characteristic red streaks, red veinbanding, and leaf distortion. Inoculated plants were not completely affected the third year after budding.

Check plants remained symptomless in both experiments.

Cross-protection studies have not been made, but field observations suggest that mosaic and shoestring are distinct from ringspot and stunt[3,4] of blueberry. All 4 diseases were common in 1 old blueberry planting, and many plants showed symptoms of 2 or 3 of the viruses in combinations. These findings indicated that 1 virus did not protect against another. No plant that showed symptoms of all 4 virus diseases was observed.

Mosaic and shoestring have been found on several varieties in all areas in New Jersey where cultivated blueberries are grown and have been observed occasionally on wild plants. Although both diseases are of minor importance at present, they are a potential threat to the industry. Varietal responses to these and other blueberry viruses are under study. Vectors are not known.—U. S. Department of Agriculture and New Jersey Agricultural Experiment Station, New Brunswick, N. J.

Stem Lesions Associated with Xanthomonas alfalfae.[1] RAYMOND D. BRIGHAM. An outbreak of bacterial leaf spot of alfalfa (*Medicago sativa* L.), incited by *Xanthomonas alfalfae* (Riker, Jones, & Davis) Dows., was observed in Iowa on June 19, 1956.[2] At first found only in experimental plots, by September the disease was observed to be present in many fields of alfalfa in the central part of the state. Damage to alfalfa was generally light, possibly because of the appearance of the disease late in the growing season. In addition to the usual leaf spotting symptoms, stem lesions also were observed to occur under field conditions.

The lesions on alfalfa stems were first noticed June 28, 1956, on a very susceptible Cossack selection at the Iowa State College Agronomy Farm, Ames, Iowa. The infected areas were small, brown to black in color, and slightly elongated. These later coalesced to form lesions varying in length from 1 to 6 cm. The stems were not girdled in most cases, but the lesions often extended ½ to ¾ the distance around the stem (Fig. 1, A). Dried exudate was sometimes present in a very thin film that peeled irregularly from the lesions. Rupturing of the epidermis within the area covered by the lesions also occurred in some cases as the disease progressed.

Broken stems were observed as a result of the weakened areas in the stem tissue. This damage was

[3] Hutchinson, M. T., and E. H. Varney. 1954. Ringspot—a virus disease of cultivated blueberry. Plant Disease Reptr. 38: 260-262.

[4] Wilcox, R. B. 1942. Blueberry stunt, a virus disease. Plant Disease Reptr. 26: 211-213.

[1] Journal Paper No. J-3080 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1265.

[2] Brigham, R. D. 1956. Bacterial leaf spot of alfalfa in Iowa. U. S. Dept. Agr. Pl. Dis. Rptr. 40: 830.

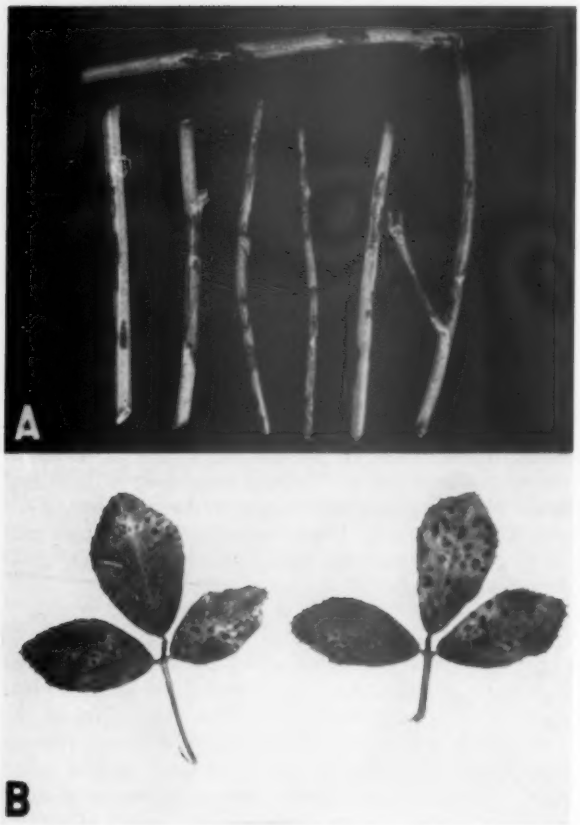

FIG. 1. Symptoms produced by *Xanthomonas alfalfae* on alfalfa. A) Stem lesions. Note broken stem. B) Leaf lesions.

observed in a first-year space-planted nursery in which plants became infected later in the season. The stems were broken over at various heights, ranging from directly above the crown to 8 in. above. In some cases, the broken portion became separated from the plant, but usually it remained attached and continued to live.

The bacterium causing the disease was readily isolated from the leaf and stem lesions, and alfalfa plants were inoculated under greenhouse conditions by applying a water suspension of the organism to the under surface of the leaves by means of a small power sprayer. Normal leaf lesions, which were small, round, water-soaked areas about 1 mm in diameter, appeared. As the spots enlarged to 2–3 mm in diameter, the centers became pale yellow, brown margins developed, and a straw-colored halo surrounded the individual lesions (Fig. 1, B). The leaflets became chlorotic and dried progressively from the tip toward the petiole; splitting of the leaflet was observed in some cases. The leaflets, when dry, fell readily from the plants. The same symptoms were observed under field conditions, and moderate defoliation was noted on susceptible plants. Stem lesions were observed under greenhouse conditions but were infrequent. The organism was reisolated from plants in the greenhouse, and successful inoculations were again made. Although a complete bacteriological characterization was not made, the cultures of the organism were similar in appearance to those described by Riker et al[3] and Patel et al.[4]

The original report of the disease by Riker et al was from an alfalfa nursery where plants had been repeatedly splashed when watered with a garden hose; however, extremely dry weather conditions had prevailed for several weeks when the symptoms of the disease were first observed at Ames. This would suggest that the bacterium was able to infect alfalfa and later be disseminated under very adverse conditions of low relative humidity and low rainfall.

The type of lesion produced on alfalfa stems under field conditions is similar in appearance at some stages to lesions produced by fungi that cause black stem, particularly *Phoma herbarum* var. *medicaginis* West. ex Rab. and *Cercospora medicaginis* Ell. & Ev. The stem-blackening symptoms induced by the different pathogens are easily confused in the field.

This is believed to be the first reported outbreak of the disease in commercial fields, and the first reported occurrence of stem lesions under field conditions.—Department of Botany and Plant Pathology, Iowa State College, Ames, Iowa.

Tomato Rosette Caused by a Virus Complex.[1] G. R. DOERING,[2] W. C. PRICE,[3] AND S. B. FENNE. The purpose of this note is to record the observation that tomato rosette disease is caused by a mixture of 2 separate and distinct viruses, rather than by a single strain of tobacco mosaic virus as originally supposed.[4] The rosette disease, which was first observed in tomato fields of southwest Virginia, is characterized by severe stunting, fernleaf, rosette, shoestring, and witch's-broom effects. Reproduction of the disease in tomato (*Lycopersicon esculentum* Mill.) plants by subinoculation from single necrotic local lesions was previously considered evidence of the single-virus nature of the infection. It must now be supposed that such lesions occasionally contained more than 1 virus.

The 2 viruses that in combination cause rosette are the tomato rosette strain of tobacco mosaic virus (TMV) and a highly instable virus, referred to in this note as the shoestring virus, not identifiable with any heretofore described. Because of its instability, this

[3] Riker, A. J., F. R. Jones, and Marguerite C. Davis. 1935. Bacterial leaf spot of alfalfa. Jour. Agr. Res. 51: 177-182.

[4] Patel, M. K., Y. S. Kulkarni, and G. W. Dhande. 1949. Bacterial leaf-spot of lucerne. Indian Phytopathol. 2: 166-167.

[1] Based on a thesis presented to the graduate faculty of the University of Pittsburgh in partial fulfillment of the requirements for the Master of Science Degree.

[2] Present address: Addison H. Gibson Laboratory, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.

[3] Present address: Citrus Experiment Station, Lake Alfred, Florida.

[4] Price, W. C., and S. B. Fenne. 1951. Tomato rosette, a severe disease caused by a strain of tobacco mosaic virus. Phytopathology 41: 1091-1098.

second virus can be removed from the complex by allowing infectious sap to remain at laboratory temperature for a few hours; the sap will then contain only the rosette strain of TMV. The latter can be removed from the complex because it is localized in *Nicotiana glutinosa* L. like other strains of TMV, whereas shoestring virus becomes systemic in *N. glutinosa*, in which it causes stunting of plants and bronzing and distortion of leaves.

Symptoms produced by the rosette strain of TMV resemble those produced by other mild strains of TMV. This strain has been differentiated from other mild strains on the bases of pH of maximum optical density[5] and serological specificity.[6]

The shoestring virus produces, in tomato, very mild mottling and slight undercurling of leaves; in Turkish-type tobacco (*N. tabacum* L.), chlorotic ringspots, fine mottling, and bronzing of leaves; and in *N. rustica* L. and in *N. sylvestris* Spegaz. & Comes, vein clearing and vein banding. Sap from tomato plants containing shoestring virus became noninfectious when allowed to stand at 20° C for as short a time as 1.5 hours; sap frozen immediately after extraction was infectious 5 days later when thawed but not when freshly thawed 5 months later.

Shoestring virus was recovering from infected tomato and tobacco plants growing in a greenhouse only during the cool months of the year; it was not recovered by rubbing other plants with sap from such plants during the warm summer months despite many attempts to do so. Plants infected during cool weather, however, carried the virus through the summer and again became sources of infection in the fall. This apparent instability of the shoestring virus component of the complex undoubtedly accounts for some of the variability in symptomatology previously reported[4]; symptoms observed were sometimes those caused by the rosette strain alone, sometimes those caused by the complex.

The shoestring and rosette components introduced simultaneously into tomato plants caused shoestring and witch's-broom effects like those previously described.[4] When both were introduced into tobacco plants, the plants became badly stunted (Fig. 1) and developed numerous strap leaves in which the blade was reduced to little more than midrib. Similar effects were induced when other strains of tobacco mosaic virus were combined with shoestring virus. These effects, in both tobacco and tomato plants, were particularly severe when Holmes' ribgrass strain of TMV was used in combination with shoestring virus; infected plants were reduced to about ¼ normal size.

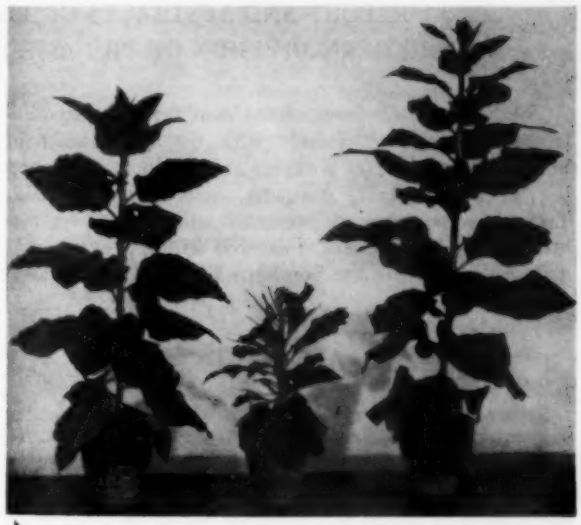

FIG. 1. Symptoms produced in Turkish tobacco plants by, left, tomato rosette strain of tobacco mosaic virus, right, the shoestring virus, and, center, a combination of both viruses.

Similar but less severe effects were induced in tomato plants by a combination of shoestring virus and ordinary cucumber mosaic virus; shoestringing, which developed in the doubly inoculated tomato plants, did not develop in plants infected with cucumber mosaic virus alone. This is in contrast to the finding of Doolittle,[7] who described shoestringing and witch's broom produced by cucumber mosaic virus alone; a simple interpretation of the discrepancy is that the shoestringing symptom can be induced by certain strains of cucumber mosaic virus but not by other strains.—Department of Biological Sciences, University of Pittsburgh, and Extension Division, Virginia Polytechnic Institute.

[5] Gordon, R. B., and W. C. Price. 1953. Differentiation of tobacco mosaic virus strains by differences in pH of maximum optical density. Archiv. Biochem. Biophys. 45: 117-123.

[6] Moorhead, Ellen L., and W. C. Price. 1953. A new serological test for tobacco mosaic virus. Phytopathology 43: 73-77.

[7] Doolittle, S. P. 1943. Tomato diseases. U. S. Dept. Agr. Farmers Bul. 1934, 83 p.

REPORT AND ABSTRACTS OF THE 1957 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The Southern Division of the American Phytopathological Society met jointly with the Association of Southern Agricultural Workers at its 54th annual meeting at Birmingham, Alabama, February 4-6, 1957. Twenty-five papers were presented and the meeting was attended by 55 members. Featured on the program was a joint session with the Southern Region of the American Society for Horticultural Science on "Disease Control of Horticultural Crops."

Officers elected for 1957 were as follows: *President*, W. W. HARE; *Vice-President*, G. B. LUCAS; *Secretary-Treasurer*, W. J. MARTIN; and *Councilor*, D. E. ELLIS.

G. B. LUCAS, *Secretary-Treasurer*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Occurrence and fungicidal control of watermelon foliage diseases in Florida. CRALL, J. M., AND N. C. SCHENCK. In spray trials made at Leesburg during 1953-1956, various fungicides were evaluated for control of 4 major watermelon foliage diseases. Downy mildew (caused by *Pseudoperonospora cubensis*) was abundant except in 1955, when it was present in trace amounts. Gummy stem blight (caused by *Mycosphaerella citrullina*) occurred in all seasons. Anthracnose (caused by *Colletotrichum lagenarium*) became well established only in 1956 following artificial inoculation. Cercospora leaf spot (caused by *C. citrullina*) was important only in 1955 and 1956. Dithiocarbamates were superior to copper fungicides in the control of these diseases except downy mildew, where both kinds were equally effective. Captan was less effective than either of the above in downy mildew control. Wettable-powder formulations of zineb and maneb were superior to their tank-mix counterparts. Copper fungicides were slightly phytotoxic, and maneb was slightly so in 1954 but not in 1955 or 1956. Captan stimulated vegetative vigor and increased melon yields in 1953 but not in later years. Zineb at 2 lb. per 100 gal. is currently recommended for control of these diseases in Florida. Trial use of maneb at 1½ lb. alternately with zineb has been suggested.

Resistance to two Meloidogyne species in breeding lines of flue-cured tobacco. DROLSOM, P. N., E. L. MOORE, AND E. E. CLAYTON. A source of resistance to root-knot nematodes in *Nicotiana tabacum*, Tobacco Introduction 706, has been known for several years, but this resistance proved difficult to utilize in the development of flue-cured tobacco varieties. A satisfactory level of resistance in breeding lines was always associated with low yield and poor type. Recently, highly resistant breeding lines derived from crosses between T. I. 706 and flue-cured tobacco were hybridized with a synthetic allo-tetraploid, *N. sylvestris-tomentosiformis*. As a result, highly resistant breeding lines with good type and satisfactory yield have been made available. Since root-knot nematodes now are classified into a number of species, it is important to know the reaction of the breeding lines to the species present in the areas where flue-cured tobacco is produced. Studies have been made to determine the reaction of these newly developed breeding lines to 5 species of *Meloidogyne*. Results from greenhouse studies indicate high resistance to *M. incognita* and to *M. incognita acrita* and susceptibility to *M. arenaria*. Results were inconclusive with respect to *M. hapla* and *M. javanica*.

Efficacy of certain cultural practices as control measures for stem rot of peanuts. GARREN, KENNETH H. A cooperative study of the effects of cultural practices on development of stem rot of peanuts caused by *Sclerotium rolfsii* is underway at Holland, Virginia, by the Horticultural Crops Research Branch of the U. S. Department of Agriculture and the Virginia Agricultural Experiment Station. In 1955, a weed-control experiment with a Virginia bunch variety compared 1) a "dinitro" herbicide + cultivation involving no soil thrown onto plants with weed control by cultivation such that soil was thrown onto the plants and 2) deep covering of surface litter with shallow covering. Infection was less and yield greater with the treatment in which no soil was thrown onto plants than with the treatment in which soil was so thrown. The differences were statistically significant at the 1% level. The test was repeated in 1956 with similar results. The 1956 results were in favor of deep covering as compared with shallow covering. In 1956, results of an additional test with Spanish peanuts suggested that 1) the "dinitro" herbicide may have some beneficial effects independent of whether or not soil is thrown on the plants and that 2) a drench of PCNB (pentachloronitrobenzene) also tends to be beneficial.

Control of sting nematodes on upland cotton by soil fumigation. GOOD, J. M., AND S. A. PARHAM. Yields of Plains cotton, a wilt-resistant variety, were significantly greater in 1956 in plots fumigated with Dowfume W-40 (41% 1,2-dibromoethane), Nemagon (1,2-dibromo-3-chloropropane), Dorlone (78% dichloropropenes; 19% 1,2-dibromoethane), or D-D (1,3-dichloropropene; 1,2-dichloropropane) than in nonfumigated plots. The soil fumigants were applied as row treatments at rates of 9.5, 0.9, 8.0, and 9.5 gal. per acre, respectively, in rows spaced 38 in. apart. Experimental plots were 1/23 of an acre; each treatment was replicated 4 times in a randomized-block design. Average seed cotton yields were 1606, 1565, 1026, 884, and 203 lb. per acre for plots treated with Dowfume W-40, Nemagon, Dorlone, and D-D and for nonfumigated plots, respectively. Yields from plots treated with Dowfume W-40 or Nemagon were significantly higher than those from the others. Ten weeks after planting, populations of sting nematode, *Belonolaimus gracilis*, were significantly lower in plots treated with Dowfume W-40 or Nemagon than in nonfumigated plots but not so in plots treated with Dorlone or D-D. When the experiment was terminated at harvest, only Nemagon-treated plots, which were almost free of sting nematodes, had significantly lower populations than did nonfumigated plots. Incidence of Fusarium wilt was correlated with yield and with control of sting nematodes 10 weeks after planting.

Inheritance of resistance of Fusarium wilt in cowpeas. HARE, W. W. The cowpea variety Iron, resistant to races 1, 2, and 3 of *Fusarium oxysporum* f. *tracheiphilum*, was crossed with Extra Early Blackeye, Purple Hull Bunch, and Mississippi Crowder, all resistant to race 1 and susceptible or tolerant to races 2 and 3. The F_1 generation of the 3 crosses, inoculated separately with races 2 and 3, was resistant. The F_2 segregated approximately 15 resistant to 1 susceptible. The F_2 of Iron × Extra Early Blackeye was grown and the progenies tested to analyze F_2 plant

reactions to the wilt organisms. Again, segregation was 15 resistant to 1 susceptible for both races 2 and 3. The data fitted the hypothesis that resistance in each case was controlled by 2 major dominant genes. A greenhouse technique for breaking down the resistance of Extra Early Blackeye to race 1 was used on the F_2 progenies. A ratio of 1 resistant, 2 segregating, and 1 susceptible resulted, suggesting segregation of a single dominant gene for resistance to race 1 from the Iron parent. With this method, the inheritance of the type of resistance (to race 1) present in Extra Early Blackeye could not be determined, nor could the presence or absence of this resistance in Iron be determined.

Leptosphaeria (*Metasphaeria*) perfect stages for *Helminthosporium turcicum and H. rostratum*. LUTTRELL, E. S. In crosses among isolates of *H. turcicum*, ascocarps were produced in culture in 4 weeks at 20–25°C on barley straw sterilized with propylene oxide and embedded in Sachs agar in Petri dishes. The black, globular, short-beaked ascocarps were 378 μ in diameter and contained numerous asci interspersed with pseudoparaphyses. Asci were clavate-cylindrical, bitunicate, 2- to 6-spored, and 176–249 $\times$ 24–31μ. Ascospores were fusoid, hyaline, 2- to 6-septate, (typically 3) constricted at the septa, and 42–78 $\times$ 13–17 μ. Single-ascospore isolates, although variable in colony characteristics, all produced conidia typical of *H. turcicum*. The perfect stage belongs in *Metasphaeria*, or in *Leptosphaeria* if these 2 genera are considered synonymous. A similar perfect stage with asci 123–162 $\times$ 25–35 μ and ascospores 39–57 $\times$ 10–14 μ was produced by isolates of *H. rostratum*, which like *H. turcicum* has a protuberant hilum at the base of the conidium, on barley grains surface sterilized with mercuric chloride and placed on the surface of Sachs agar plates. Single-ascospore isolates produced the conidial stage. In *H. rostratum*, ascocarp production was exceedingly sparse and inconsistent.

Suitability of soybeans var. Ogden and twelve other plants as hosts of the spiral nematode. MINTON, NORMAN A., AND ELDON J. CAIRNS. Various crop plants and weeds frequently found in Alabama where *Helicotylenchus nannus* Steiner 1945 occurs were tested in the greenhouse as hosts. Three soybean plants were grown for 120 days in each of 40 8-in. pots containing sterilized soil. Twenty of these were infested with 500 spiral nematodes each at time of planting and 20 were not. Five additional pots kept free of vascular plants were infested with 500 spiral nematodes each. The dry weight of roots of inoculated plants was less (statistically significant at the 5% level) than that of the controls. The difference between the seed yields of the inoculated and non-inoculated plants was not significant. The nematode population had multiplied by an average of 10.6 in pots containing soybean plants, whereas an average of only 15 nematodes per pot survived in the absence of vascular plants. The spiral nematodes were found to multiply in similar greenhouse tests with the following 12 kinds of plants: cotton, Ladino clover, oats, grain sorghum, Dallis grass, Bermuda grass, nut grass, Johnson grass, fescue, Sweet Sudan, and orchard grass. Under the condition of the experiment, *H. nannus* was not found to be visibly pathogenic on any of the plants tested except soybean var. Ogden. Root penetration by the nematodes occurred only in fescue.

Segregation for flavor in disease-resistant lines of flue-cured tobacco. MOORE, E. L., AND P. N. DROLSOM. An objectionable flavor of smoke, described as "cigar or piney," has been reported in disease-resistant lines of flue-cured tobacco from a cross of Dixie Bright 102 $\times$ Hicks. One of these lines, F6 3006-5, was used as the seed parent in a cross with an F_2 having resistance to bacterial wilt (caused by *Pseudomonas solanacearum*), black shank (caused by *Phytophthora parasitica* var. *nicotianae*) and root knot (caused by *Meloidogyne* spp.). The off-flavor found in 3006-5 was detected by a manufacturer's smoke panel in unaged leaf from individual plants selected at random from this F_1. Three different crosses involving parents resistant to bacterial wilt and to black shank were used in an experiment on flavor of unaged leaf from F_2 plants. The crosses were 1) normal (4009) $\times$ abnormal (3006-5); 2) normal (4009) $\times$ normal (Coker 139); and 3) normal but poor taste (4003) $\times$ normal (Coker 139). One plant from the first cross was normal, and 19 had abnormal flavor ranging from faint to strong; 9 plants from the second cross had fair and 1 good flavor; 3 plants from the third cross had poor, 9 fair, and 3 good flavor.

Parasitic nematodes in relation to the peach replant problem in Ontario. MOUNTAIN, W. B., AND H. R. BOYCE. A survey of approximately 10% of all the peach orchards in southwestern Ontario for plant parasitic nematodes showed that probably only *Pratylenchus penetrans* can be correlated with the occurrence of this problem. In field experiments involving soil fumigation, increased growth of peach was associated with the control of *P. penetrans*. When control was inadequate, or when this nematode was not present, the growth data were erratic. Soil fumigation with D-D (1,3-dichloropropene; 1,2-dichloropropane) at high dosage (40 Imperial gal. per acre) continued to give almost complete control of nematodes 2 years after application. Surveys have shown that the soil in almost all commercial peach nurseries in the province is infested with this nematode. Pathogenicity studies are incomplete, but present indications are that it will be possible to obtain convincing evidence of the depressive effects of *P. penetrans* upon the growth of peach.

The genetics of compatibility in Cochliobolus heterostrophus. NELSON, R. R. Previous studies have demonstrated that *Cochliobolus heterostrophus* (*Helminthosporium maydis*) is heterothallic and that monoconidial isolates represent 2 distinct compatibility groups. The present investigation was undertaken to determine the number of genes governing compatibility. Single-ascospore cultures were obtained from crosses of conidial isolates. Each ascospore isolate then was mated back to both conidial parents to determine its compatibility group. A total of 436 ascospore isolates obtained from 39 different conidial matings were analyzed. The number of ascospores obtained from a single cross ranged from 1 to 112. Two-hundred eleven ascospore isolates belonged to one compatibility group and 225 to the other. Preliminary studies with ascospore isolates mated back to ascospore parents have given similar ratios. These results strongly indicate that compatibility in *C. heterostrophus* is governed by a single major gene locus.

Studies on the Striga parasite of corn. NELSON, R. R., AND HOWARD R. GARRISS. *Striga asiatica* is a parasitic flowering plant that causes severe damage to the roots of many species of cultivated and wild grasses in tropical and subtropical countries of the Eastern Hemisphere. Its recent discovery on corn in North Carolina is the first known report of its presence in the Western Hemisphere. In preliminary surveys, the parasite has been found on corn and crab grass in widely scattered areas in 4 counties in southeastern North Carolina and in several adjoining counties in South Carolina. Botanically, the species is characterized by scarlet or red flowers, square aerial stems, small elongated leaves, opposite lower leaves, alternate upper leaves, and minute striated seed borne in capsules. Studies indicate that the parasite attacks a variety of economic and wild hosts that occur commonly in the sandy soils of the Coastal Plain. A small percentage of Striga seed germinates without a dormancy period, although maximum germination probably is not attained without such a period. In the laboratory, Striga seed germinated when kept in the presence of corn roots or in corn root leachates. Seed also

germinated to a limited extent in the absence of host stimuli when subjected to either scarification or alternate freezing and thawing.

The relationship of nematocides, dosage, carrier, and soil types to the control of root knot in sweetpotato. NIELSEN, L. W., AND J. N. SASSER. Dowfume W-85 (1,2-dibromoethane) and D-D (1,3-dichloropropene; 1,2-dichloropropane) were applied in the row for control of root knot (caused by *Meloidogyne incognita acrita*) in sweetpotato production. In a factorial experiment conducted for 2 seasons, the nematocides were applied at 0, ½, 1, and 2 times the recommended dosages of 10 gal./acre for D-D and 4½ gal./acre for Dowfume W-85. The fumigants were applied at these dosages in 2 forms: liquids and impregnated on vermiculite. The experiments were located on light, intermediate, and heavy soils infested with root knot nematodes. All treatments involving fumigants resulted in greater yields (than in nontreated plots) in light and intermediate soils but not in heavy soil. The fumigants impregnated on vermiculite were more efficient at the lower dosages than were the comparable liquid treatments, but they adversely affected yields at the high dosage. This effect on yield was most serious in heavy soil. Regardless of soil type, there was less cracking of sweetpotato roots from treated plots than of those from untreated plots.

Anthracnose of azaleas. STATHIS, P. D., AND A. G. PLAKIDAS. An apparently undescribed disease, characterized by very numerous, small, brown, mostly circular spots on the leaves and by severe defoliation occurred in epidemic form on azaleas in Louisiana in 1954-1956. Both Indian and Kurume varieties are affected. A species of *Colletotrichum*, with conidia typical of *Glomerella cingulata*, is found in constant association with the disease, and the disease has been repeatedly reproduced by inoculations with pure cultures of this fungus. The disease was also reproduced by inoculations with a pure culture of Colletotrichum isolated from bitter rot lesions on crabapple fruit. No sporulation occurs on the lesions while the leaf is alive, but conidia develop abundantly in acervuli on the abscised leaves. A comparison of the Louisiana disease with that caused by a specimen of *C. azaliae* Ell. & Ev. showed that the 2 diseases are different. Inoculation tests showed small differences in susceptibility among varieties. Limited tests with fungicides indicate that both copper and organic fungicides are effective in controlling the disease.

Status of sweetgum blight, 1956. TOOLE, E. RICHARD. Sweetgum blight, a disease of unknown cause, has been under observation in the Mississippi Delta during the past 4 years. The disease is characterized by dying of branches from the tip and sometimes by death of the tree. A high percentage of the fine roots are dead on diseased trees. The progress of the disease in selected locations has shown continued intensification. Mortality in 8 areas has varied from 0 to 67%, with an average of 16% after 4 years. No organism that seems likely to be the cause of blight has been isolated from the tops or roots. Injections with minor elements have had no effect on the blight. Preliminary soil studies have indicated that blight is worse on those bottomland soils that have properties contributory to a shortage of soil moisture.

LISTING OF BOOKS

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on pathological subjects. A brief desription of the contents of the books will be included.

CAMPBELL, T. H. 1956. **Plant pathology outlines.** Published by the author. Knoxville, Tennessee. Price $2.50.

These outlines, printed by the off-set duplicating process, are in a loose-leaf arrangement to facilitate selection of certain ones for laboratory exercises and insertion of class notes and discussions. An outline for the study of a parasitic plant disease is presented. This is followed by eparate laboratory outlines for some 35 plant diseases. Each laboratory outline consists of a list of materials, a set of questions, a list of basic references, and a list of additional references. With a few exceptions, each is illustrated. The appendix contains a listing of other references on general and special topics.

TISDALE, S. L., AND W. L. NELSON. 1956. **Soil fertility and fertilizers.** The Macmillan Co., New York. 430 p. Price $7.75.

After a brief historical introduction, several chapters are devoted to the elements required in plant nutrition, their role in plant growth and development, and the soil reactions that affect the availability of these nutrients. The next several chapters contain information on the manufacture, properties, and agronomic values of fertilizers and fertilizer materials. The last several chapters cover the subject of soil fertility evaluation and the use of fertilizers in a sound management program.

REPORT OF THE 48TH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The American Phytopathological Society held its 48th Annual Meeting on December 5–8, 1956, at the Netherland-Hilton Hotel, Cincinnati, Ohio. The meeting was held in conjunction with the annual meeting of the Potato Association of America. There were 659 registered in attendance. The Council met on December 5, 6, and 8, and general business meetings were held on December 6 and 8. A total of 250 contributed papers of original research were presented by members. Ten invitational papers were presented, 5 at the Fungicide Colloquium and 5 at the general symposium on Foreign Plant Quarantines and Regulatory Administration. Informal conferences were held on Diseases of Ornamental Plants, Extension, Diseases of Shade Trees, Seed Treatments, Tomato Disease Screening, and Oak Wilt. The annual dinner was held Friday evening, December 8, with about 335 members attending.

Organization of American Phytopathological Society, 1957

Council:

George W. Fischer, *President;* State College of Washington
Paul R. Miller, *President Elect;* U. S. Department of Agriculture
Helen Hart, *Retiring President;* University of Minnesota
Glenn S. Pound, *Vice President;* University of Wisconsin
William B. Hewitt, *Secretary* (3 yrs., expires 1959); University of California
Saul Rich, *Treasurer and Business Manager of Phytopathology* (3 yrs., expires 1958); Connecticut Agricultural Experiment Station
A. F. Ross, *Editor-in-Chief of Phytopathology* (3 yrs., expires 1957); Cornell University
Roy A. Young, *Councilor-at-Large* (2 yrs., expires 1958); Oregon State College
S. E. A. McCallan, *Councilor-at-Large* (2 yrs., expires 1957); Boyce Thompson Institute
John W. Heuberger, *Councilor of Potomac Division;* University of Delaware
D. S. Welch, *Councilor of Northeastern Division;* Cornell University
W. H. Sill, Jr., *Councilor of North Central Division;* Kansas State College
D. E. Ellis, *Councilor of Southern Division;* North Carolina State College
Roderick Sprague, *Councilor of Pacific Division;* State College of Washington

Representatives:

A.A.A.S. Council. J. G. Horsfall (2 yrs., expires Dec. 31, 1957), H. Rex Thomas (2 yrs., expires Dec. 31, 1958)
A.I.B.S. J. G. Dickson (4 yrs., expires June 30, 1958)
Agricultural Research Institute, National Research Council. C. L. Lefebvre; E. L. LeClerg, Alternate
Division of Biology and Agriculture, National Research Council. S. J. P. Chilton (3 yrs., expires June 30, 1958)
Food Protection Committee, Food and Nutrition Board, National Research Council. J. C. Dunegan
Board of Editors, American Journal of Botany. A. J. Riker (3 yrs., expires 1957)
American Type Culture Collection. H. H. McKinney; J. W. Gerdeman, Alternate
Associates Food and Container Institute. G. B. Ramsey
Biological Stain Commission, Board of Trustees. F. P. McWhorter
Policy Committee for Scientific Agricultural Societies. Carroll E. Cox
American Standards Association Sectional Committee on Common Names for Pest Control Chemicals, K62. S. E. A. McCallan; W. D. McClellan, Alternate

Standing Committees:

Extension
Arden Sherf, Chr.; Gordon Brandes, H. J. O'Reilly, Harlan Smith, M. C. Shurtleff, Earl Wade, J. C. Wells
Finances and Investments
A. E. Dimond, Chr.; C. L. Lefebvre, R. H. Wellman, Saul Rich (*ex officio*)
History of Plant Pathology
J. A. Stevenson, Chr.; J. Walter Hendrix, J. G. Leach, E. L. LeClerg, Karl Maramorosch
International Cooperation
E. C. Stakman, Chr.; A. A. Bitancourt, G. H. Coons, Carlos Garces, W. F. Hanna, J. G. Harrar, Lee Ling, J. A. B. Nolla, J. W. Oswald, H. A. Rodenhiser, J. A. Stevenson, Freeman Weiss, F. L. Wellman
Phytopathological Classics
L. C. Knorr, Chr.; A. F. Ross (*ex officio*), J. C. Walker, Paul H. Wooley
Phytopathological Monographs
G. C. Kent, Chr.; Arthur Kelman, Seth Locke
Phytopathological News Page
K. W. Kreitlow, Chr.; M. W. Cormack, Spencer H. Davis, D. E. Ellis, Shirl Graham, L. O. Weaver, Roy Wilcoxson
Phytopathological Reviews
Russell Stevens, Chr.; J. R. Shay, W. H. Sill, Jr.
Placement
Earle Hanson, Chr.; W. E. Cooper, H. Rex Thomas
Public Relations
P. R. Miller, Chr.; Paul Hoffman, T. H. King, G. K. Parris, R. A. Young, G. A. Zentmyer, John L. Weihing
Regulatory Work and Foreign Plant Diseases
G. L. Stout, Chr.; J. G. Dickson, E. C. Blodgett, E. G. Rex, W. H. Wheeler, R. P. White, W. C. Snyder, Gerald Thorne
Seed and Plant Material Certification
K. F. Baker, Chr.; John Hardison, C. H. Arndt, Wayne Bever, L. C. Cochran, D. J. Hagedorn, W. H. Wheeler

Special Committees:

Fungicide Colloquium
L. G. Utter, Chr.; Curt Leben, John Rowell, D. L. Stoddard, D. F. Crossan, W. A. Kreutzer, J. E. Mitchell
Industrial Relations
E. E. Feichtmeir, Chr.; C. J. Krister, J. S. Tidd, R. H. Wellman, S. G. Younkin, L. G. Utter
Advisory Committee on Nematology
J. N. Sasser, Chr.; M. B. Linford, Harold Jensen, W. J. Martin, J. A. Pinckard
Membership
Glenn S. Pound, Chr.; Robert Campbell, R. G. Grogan, J. E. Kuntz, W. J. Hooker, Arthur Kelman, D. A. Roberts, J. R. Shay, P. M. Simmonds, Saul Rich (*ex officio*), J. E. Thomas
Teaching of Plant Pathology
W. H. Bragonier, Chr.; D. C. Arny, C. W. Ellett, R. J. Green, Jr., Richard Kiesling, George Nyland, J. H. Owen

Utilization of Plant Pathologists and Facilities in National Emergenies
H. H. THORNBERRY, Chr.; A. L. ANDERSON, H. T. COOK, M. F. KERNKAMP, C. J. KINGSOLVER, J. G. LEACH, C. L. LEFEBVRE, H. REX THOMAS
Virus Type Culture Collection
H. H. MCKINNEY, Chr.; R. W. FULTON, T. J. GRANT, ELLEN MOORHEAD, M. P. BRAKKE

Temporary Committees:

Advisory Committee on Collecting and Disseminating New Fungicide Data
L. G. UTTER, Chr.; J. W. HEUBERGER, P. R. MILLER, R. H. WELLMAN
Advisory Committee on Integration of Research on Soil-Borne Pathogens
W. C. SNYDER, Chr.; J. O. ANDES, L. W. KOCH

REPORTS OF OFFICERS AND COMMITTEES

Report of the Secretary. On November 15, 1956, the American Phytopathological Society had 1817 members, including 113 Life Members, 17 Emeritus Members, 3 Emeritus and Life Members, 13 Sustaining Associates, and 1671 Regular Members. Of the 1671 Regular Members, 146 are New Member Applicants of which 85 are students. Since our last meeting, word has been received of the death of the following members or former members: H. L. Bolley, Charter Member; Nellie A. Brown, Charter Member; B. M. Duggar, Charter Member; George M. Reed, Charter Member; Harry B. Shaw, Charter Member; C. L. Shear, Charter Member; E. E. Honey, F. R. Jones, E. M. Gilbert, W. A. Jenkins, A. J. Mix, Fred M. Rolfs, C. A. Schwarze.

GLENN S. POUND, *Secretary*

Report of the Treasurer.

Receipts:		
Balance on hand, July 1, 1955	$ 7,309.44	
Membership Dues	2,748.00	
Sales of Fungicide Data Summaries	433.00	
Total Receipts		$10,490.44
Expenditures:		
Office of Secretary	499.80	
Office of Treasurer	330.98	
Expenses, Placement Committee	44.88	
Expenses, Annual Meeting (Atlanta)	831.69	
Expenses, Publication of Fungicide Data Summaries	666.71	
Expenses, Public Relations Committee	474.00	
Dues, A.I.B.S.	2,235.00	
Expenses, Membership Drive	282.32	
Contribution to Joint Committee on Grassland Farming	10.00	
Contribution to American Type Culture Collection	500.00	
Contribution to Biological Division, NAS, NRC	100.00	
Refunds	2.00	
Total Expenditures		5,977.38
Balance on hand, June 30, 1956		$ 4,513.06

SAUL RICH, *Treasurer*

Report of the Business Manager of *Phytopathology*

Receipts:		
Balance on hand, July 1, 1955	$13,223.29	
Subscriptions and Member-subscriptions	25,227.41	
Advertisements	3,567.80	
Sales of Back Issues	1,386.80	
Reprints and Excess Illustrations	5,250.68	
Sustaining Associate Memberships	1,200.00	
Interest on Investments	982.34	
Sales of Investments	1,737.50	
Total Receipts		$52,575.82
Expenditures:		
Manufacture and Distribution of PHYTOPATHOLOGY	$18,328.92	
Cost of Reprints	2,004.43	
Office of the Business Manager	1,007.21	
Office of the Editor-in-Chief	3,178.13	
Office of the Advertising Manager	430.92	
Cost of New Investments	2,093.86	
Collector of Internal Revenue	1,256.64	
Newsletter	8.64	
Purchase of Back Issues	10.00	
Refunds	87.00	
Total Expneditures		28,405.75
Balance on hand, June 30, 1956		$24,170.07

SAUL RICH, *Business Manager*

Invested Funds of the American Phytopathological Society and *Phytopathology*. Amounts are as of June 30, 1955. Dividends and interest are for fiscal year, July 1, 1955, to June 30, 1956.

Sinking Fund:	
U. S. Savings Bonds, Series J (at maturity)	$ 2,500.00
U. S. Savings Bonds, Series F (at maturity)	425.00
Northwestern Fed. Sav. & Loan Assn. (certificate) (Div. 65.00)	2,000.00
Family Finance Corp., common, 7 sh at par (Div. 10.51)	7.00
Westinghouse Electric Corp., 22 sh at cost (Div. 44.00)	849.08
Standard Oil of Ohio, common, 60 sh at cost (Div. 127.50)	2,018.65
American Cyanamid Co., common, 40 sh at cost (Div. 85.00)	2,160.47
Johns-Manville Corp., common, 50 sh at cost (Div. 118.75)	1,664.27
New Haven Savings Bank, on deposit (Int. 5.98)	204.05
Total	$11,828.52
Lyman Memorial Fund:	
Westinghouse Electric Co., common, 35 sh at cost (Div. 70.00)	$ 1,350.81
American Tel. & Tel., common, 18 sh at cost (Div. 148.40)	2,784.33
Total	$ 4,135.14
PHYTOPATHOLOGY *Contingency Fund:*	
Johns-Manville Corp., common, 14 sh at cost (Div. 33.25)	$ 466.00
Arlington & Fairfax Sav. & Loan Assn. (Int. 216.02)	7,363.25
Prudential Bldg. Assn. (Int. 218.72)	6,413.57
Arundel Fed. Sav. & Loan Assn. (Int. 286.68)	8,407.12
Southern Fed. Sav. & Loan Assn. (Int. 305.40)	8,955.33
Decatur Fed. Sav. & Loan Assn. (Int. 276.48)	8,107.74
Fidelity Fed. Sav. & Loan Assn. (Int. 256.96)	7,535.42
Southwest Sav. & Loan Assn. (Int. 251.45)	7,373.95
Lawn Sav. & Loan Assn. (Int. 280.00)	8,000.00
Total	$62,622.38

SAUL RICH, *Treasurer and Business Manager*

Report of the Auditing Committee. On September 10, 1956, an examination was made of the books of the American Phytopathological Society and of PHYTOPATHOLOGY for the period July 1, 1955, to June 30, 1956. All receipts, expeditures, and funds were found to be accurately

recorded and in order.

The committee observed that the 1955–56 expenditures were only about 10 per cent greater than the 1954–55 expenditures. We feel that this indicates an insufficient increase in activity by the Society, despite its excellent financial condition and expressed desire by the membership for more activity.

A. W. Feldman
Patrick M. Miller
Paul E. Waggoner

Report of the Advertising Manager. From October, 1955, through September, 1956, there were 84 advertising pages with 100 paying advertisements. The gross income was $3927.84 with a balance of $3748.76 after agency commissions and cash discounts were deducted. Expenses of the advertising manager were $28.39 for postage, telephone and telegraph charges, and second sheets, and $72 for stenographic assistance. The commission to the advertising manager amounted to $324.87, making a total of $425.26 in expenses.

Total income to the Society is $3323.50.

Leonard J. Alexander, *Advertising Manager*

Report of the Chairman of *Phytopathological Classics*. A plan for the reorganization of the affairs of Phytopathological Classics was presented to the Council at its last annual meeting, and in consequence, the Society took action to place all activities of the enterprise under a single standing committee. It was further arranged that the various duties involved in the operation of the Classics be delegated by a chairman to members of the PC Committee. Accordingly, Dr. Paul H. Wooley was asked to serve as Treasurer, and in that capacity to be in charge of the distribution of publications and of the collecting and accounting of funds.

The Treasurer's financial report for the fiscal year 1955–1956 follows.

Receipts:

Cash balance on July 1, 1955			$ 1,516.69
Received during fiscal year 1955–56			187.00
From sale of Classics	$172.50		
Originals	$141.00		
Reprints	31.50		
Accounts receivable*		14.50	
Originals	11.50		
Reprints	3.00		
Total receipts			$ 1,703.69

Disbursements:

Postage	34.00	
Printing letterheads and postcards	19.25	
Advertising	2.16	
Cash balance on June 30, 1956	1,648.28	
Total disbursements		1,703.69

**Over the years various amounts under Accounts Receivable have proved to be uncollectable. The amounts, totalling $25.50, have been written off as bad debts and do not appear in this statement.*

The number of copies of Phytopathological Classics sold during the fiscal year was 174, having a total value of $187.00. Number of Classics on hand June 30, 1956, in Ithaca, N. Y., and in Lake Alfred, Fla.: No. 1, 497; No. 2, 497; No. 3, 22; No. 4, 107; No. 5, 329; No. 6, 375; No. 7, 318; No. 8, 497; No. 9, 712.

The above accounting, with its favorable cash balance, indicates that consideration can now be given to 1) reprinting PC No. 3, the original edition of which is now nearly exhausted, and to 2) publishing a new title in the PC series, which will become PC No. 10. Tentative arrangements are to feature the two classic papers of Agostini Bassi, originally published in 1835 and 1836 under the title "Del mal del signo." Dr. G. A. Ainesworth of the University of Exeter has consented to make the translation from the Italian. It would be fitting that this rendition into English be made this year in celebration of the death of Bassi who, important though he is in the development of ideas concerning pathogenesis, is but little known in this country.

L. C. Knorr, *Chairman*

Report of the Committee on Finances and Investments. Our total invested funds of $78,586.04 earned $2,800.10 in the year ending June 30, 1956, a yield of 3.56 per cent. This is to be compared with a yield of 3.4 per cent on $74,783.58 in the previous year. The slightly improved yield was due entirely to increased dividends and interest on our insured savings and loans accounts as a result of shifts in accounts made during 1955.

The following table summaries our investments and their earnings for the past fiscal year.

Investment	*Total*	*Earnings*	*Yield*
Insured Savings & Loan Accounts	$64,156.38	$2,156.71	3.36%
Common stock (original cost)	11,300.61*	637.41	5.64%
U. S. Savings Bonds, Series J, at maturity	2,500.00		
U. S. Savings Bonds, Series F, at maturity	425.00		
New Haven Savings Bank account	204.05	5.98	3.00%
Totals and average yield	$78,586.04	$2,800.10	3.56%

**At the close of the market on September 17, 1956, our common stock holdings were worth $15,797.80.*

During the year, the following transactions were conducted: 1) Cashed $1,725.00 in matured U. S. Savings Bonds; 2) Bought $2,500.00 worth of U. S. Savings Bonds, Series J; 3) Bought 2 shares of AT&T common stock for $146.93 per share with rights during year; 4) Received 32 shares of Johns-Manville common stock in a 1-for-1 split; 5) Received 10 shares of Standard Oil of Ohio common stock in a 1-for-5 split; 6) Dividends and interest from Savings and Loan pass book accounts and New Haven Savings Bank account were left to accrue and are included in totals given in summary.

The Society is in a tax category which allows a $1,000 exemption for unrelated business income. Any amount in excess of $1,000 (up to $25,000) is taxed 30 per cent. Total income on investments exceeded the exemption by $1,800.10, which means we have a tax obligation of $480.00.

Further details are in the Chairman's hands and will be furnished upon request.

R. H. Wellman, *Chairman*

Report of the Editor-in-Chief. During 1956, the Editorial Board recommended and the Council adopted 2 changes in rules concerning acceptance of papers for publication in Phytopathology. One of these enables subscribing institutions in foreign countries to transmit manuscripts by nonmembers for publication in Phytopathology. The other makes possible the acceptance of a limited number of Phytopathological Notes not based on original research. These new rules were publicized in the News section of the June issue and are stated in the standing material printed each month in Phytopathology. Nevertheless, no great influx of new manuscripts has resulted; to date, the only paper accepted under these new rules is a Phytopathological Note on a matter of historical interest.

Volume 46 contains 696 pages of technical material, 8.5 pages of News, 90.5 pages of advertising matter, a 1-page drawing guide, an index, and a list of errata. Pages 411 and 412 are nonexistent because of an error in pagination. The technical material consists of 142 articles; 47 Phyto-

pathological Notes; a commemorative resolution in recognition of the 200th anniversary of the birth of Prevost, together with a biographical sketch of his life and an evaluation of his Memoir on bunt; 6 obituaries; the report and 183 abstracts of the 47th annual meeting of the American Phytopathological Society; the report and 17 abstracts of the 11th annual meeting of the Northeastern Division; the report and 28 abstracts of the 13th annual meeting of the Potomac Division; the report and 44 abstracts of the 38th annual meeting of the Pacific Division; a notice for the Program Committee for the 50th anniversary of the American Phytopathological Society; and 8 book listings.

As of November 12, the December issue and the 250 annual abstracts, which will comprise ⅔ of the January issue, have been completed except for final printing, and 87 manuscripts are in the process of being edited. Of these, 4 are in this office, 9 have been sent to reviewers, 24 have been returned to authors for revision, 27 are at the printer's, and 23 are in galley proof. From November 30, 1955, to November 12, 1956, 11 manuscripts have been rejected by the editors or withdrawn by the authors. Of the 24 returned to the authors for changes, 11 have been held by the authors for from 6 months to 2 years; presumably, most of these have been withdrawn without notification.

Again it is a pleasure to express my appreciation to the Editors and Associate Editors for their excellent work; to the Business Manager and the other officers of the Society for their support and efficiency; to Mr. John Ferguson, 3rd, and staff of the Monumental Printing Company for the quality of their work and for their unfailing cooperation in meeting each new problem as it arises; to Mrs. Carrol E. Cox for her competent indexing of Volume 46, and to all contributing authors for sending manuscripts and for their quite general acceptance of the editing in the spirit in which it was intended. Finally, I am pleased to acknowledge the continued financial support of the Sustaining Associates of the Society.

A. F. Ross, *Editor-in-Chief*

Report of the Editor of News. At the last annual meeting, the Council of the American Phytopathological Society authorized formation of a Standing Committee for preparation of the News page in PHYTOPATHOLOGY. The present editor of News was designated chairman of the Committee. The Council selected as committee members a regional representative from each of the geographical divisions of the society. The members of the committee consisted of L. O. Weaver, College Park, Maryland; Shirl O. Graham, Pullman, Washington; D. E. Ellis, Raleigh, North Carolina; S. H. Davis, New Brunswick, New Jersey; J. F. Schafer, Lafayette, Indiana; and M. W. Cormack, Lethbridge, Alberta, Canada. Immediately following formation of the committee, the chairman contacted each member of the committee and suggested the area by states within which each member of the committee would have responsibility. It was suggested that each committee member break down his territory further by designating a State representative, and if there was more than one institution within a State that had a Department of Plant Pathology, the State representative should in turn designate an individual at each of these institutions. Within such an organizational structure, we should have listening posts thoughout the United States and Canada for News items. Each member of the committee was asked to send items that came to hand into the Office of the Editor no later than the 18th of the month preceding publication. In this way, all items could be assembled and prepared for the printer. Most of the members of the committee have cooperated exceedingly well, and we have been supplied with adequate News items. As a result, the News page in the Journal has been expanded to one full page of items every other month with a half page of news in alternate issues. It is hoped that perhaps some time in the near future a full page of news items can be prepared each month.

K. W. KREITLOW, *Chairman*

Report of the Committee on Donations and Legacies. Continuing the precedent set, the following gratuitous subscriptions to PHYTOPATHOLOGY are recommended for renewal in 1957. They are Amer. Inst. of Biol. Sciences, % The Managing Editor, AIBS Bulletin, Washington 25, D. C.; Wm. B. Brierly, Univ. of Reading, Reading, England; Centro de Documentacion Cientifica y Tecnica, Dr. A. Perez Vitoria, Director, Plaza de la Ciudadela 6, Mexico, D. F.; The Jewish Nat'l. and Univ. Library, C. Wormann, Director, P. O. Box 503, Jerusalem, Israel; K. Minev, Faculty of Agric., Skopje, Macedonia, Yugoslavia; H. Pape, Schlosskoppelweg 8, Kitzeberg bei Kiel, Brit. Zone, Germany; Jaroslav Peklo, Chodska 3", Prague XII, Czechoslovakia; Permanent Book Univ. Exhibition, Postbox 520, Zagreb, Yugoslavia; Prof. J. A. Sarejanni, Director, Inst. Phytopath. Benaki, Kiphissia, Athens, Greece.

FRANK L. HOWARD, *Chairman*

Report of Committee on International Cooperation. The Committee has considered a number of projects by correspondence, and several of the members have met on an opportunistic basis at various times. Although nothing spectacular has been accomplished, various members have helped to aid in a number of worthwhile activities.

The Committee has encouraged people in a number of foreign countries to contribute to the FAO Plant Protection Bulletin and has had enthusiastic avowals of support and promises to contribute.

In trips to Pakistan, Lebanon, Germany, and Mexico, the chairman made particular effort to help facilitate rapid exchange of literature and communications between organizations and individuals with common interests. Other members of the Committee in the course of their travel have functioned similarly.

Four Committee members—G. H. Coons, W. F. Hanna, H. A. Rodenhiser, and E. C. Stakman—attended the Third International Wheat Rust Conference in Mexico in March, 1956. They participated in the scientific activities and attempted to help formulate concrete plans for cooperation on the wheat rust problem.

Steps have been taken also to bring about closer collaboration with a number of phytopathologists in Germany, as relations have not yet returned to normal since the war.

Encouragement has been given wherever possible to cooperative testing and disease resistance on a regional basis. It is hoped that this can be promoted still further.

One project that has been suggested and which will be brought before the Committee at the earliest opportunity is the proposal to try to find ways and means of making sure that PHYTOPATHOLOGY is available in all important libraries in certain countries where pathological work is either languishing or just beginning.

E. C. STAKMAN, *Chairman*

Report of Placement Committee. Fifty-two plant pathologists, 1 horticulturist, 1 botanist, and 1 bacteriologist had applications in the active files of the Placement Committee during 1956. Seventeen of the applicants were placed during the year, 1 withdrew her application, 13 had their applications placed in the inactive files because they could not be contacted, and 24 are currently being considered for positions. The minimum salary requested by applicants ranged from $4,000 to $15,000. Six of the applicants were women, and 49 were men. Age of applicants ranged from 26 to 60 years, and all except 5 were citizens of the United States.

Applications were sent to 46 prospective employers in an attempt to fill 57 positions. These positions were of the following kinds: 15 with the U. S. Department of Agriculture, 16 with state agricultural experiment stations or universities, 5 with teachers' colleges, 6 foreign assignments, and 15 with commercial firms. From 1 to 12 applications were submitted to each prospective employer; the total number of applications mailed was 239.

It is the responsibility of the applicants to keep the Committee informed of changes in address or employment status. If after 3 attempts the Committee does not receive a reply from an applicant, his application is placed in the inactive files. At the beginning of the year attempts were made to contact all applicants, and the files have been brought up to date.

Persons interested in federal positions should fill out Standard Form 57 and mail it to the U. S. Civil Service Commission, as it is becoming increasingly difficult to place persons in federal service unless their names are included on a Civil Service roster.

E. W. HANSON, *Chairman*

Report of the Committee on Public Relations. The work of this Committee has continued on the lines initiated two years ago, namely the publicizing of some of the outstanding plant disease research papers. A series of articles has been prepared and published in the major farm magazines. Robert Rathbone, our writer, has been able to present certain important phases of our plant pathological activities through the Department official channels. Press releases of about 20 papers have been prepared and will be released at the time of their presentation at Cincinnati. We also expect to have our Public Relations man available at these meetings this year to make proper contact with the various news agencies. Again, good coverage of material published in PHYTOPATHOLOGY has resulted through the courtesy of the Editor, who has regularly made galleys available to us prior to publication.

For increased effectiveness of our program we again solicit the cooperation of our Society members, who can help by submitting their articles to our Committee when they are accepted for publication.

PAUL R. MILLER, *Chairman*

Report of the Committee on Regulatory Work and Foreign Plant Diseases. The Committee held 3 meetings during the annual meeting of the Society at Atlanta, Georgia, to discuss and act upon the following items:

1) Revision of Section 3 of the resolution "Defining the Responsibilities, Functions, and Procedures of the Committee" as requested by the Council. The revision was resubmitted to the Council and approved by the Council at its final meeting. Copies of the revised resolution are on file with the secretary of the American Phytopathological Society, and a copy was transmitted to the Plant Quarantine Branch of the U. S. Department of Agriculture.

2) Two items referred to the Committee were acted upon. a) Evaluating the danger of importing new and dangerous pathogens in and on seed sent into the country and possible safe means of treatment. A joint study of the problem with the Committee on Seed and Plant Material Certification was suggested at the time of the next annual meeting, and tentative arrangements have been made. b) A requested study of present quarantine procedure used by the Plant Quarantine Branch, U. S. Department of Agriculture, in regard to nematodes in incoming shipments. Present procedure is to consult with individual states in instances where imported plant materials are consigned to individuals within the state. State requirements vary widely. Committee suggested study of nematode problem be referred to recently formed Advisory Committee on Nematology of the Society.

A preliminary study and discussion were made of regulations incorporated into Senate Bill 3952 "To regulate the movement from foreign countries into and through the United States and the interstate movement, of plant pests, and for other purposes." Mimeographed copies of the Bill were furnished the Committee later for further study.

The Committee instructed the Chairman to petition the American Phytopathological Society for a Symposium on Foreign Plant Quarantine and Regulatory Administration to be presented as a part of the program of the Forty-eighth Annual Meeting. A timely program designed to give all plant pathologists the latest information on plant quarantines and the procedures to follow in securing quick movement of pathogens and disease specimens necessary in research programs appeared especially important, with Bill S3952 before Congress.

J. G. DICKSON, *Chairman*

Report of the Committee on the History of Plant Pathology. The Committee on the History of Plant Pathology has followed the original directive received from the Council of the Society and has carefully conserved all pertinent materials that have been offered or otherwise acquired. In particular, Dr. Maramorosch has given a set of prints from his collection of portraits of plant pathologists, and Dr. Leach has turned over items from the files of the late C. R. Orton. Society records referred to in last year's report are being combed for relevant material.

It occurs to the Committee that materials already in hand may serve a useful purpose in the preparation of any historical account which may be planned for the 50th anniversary celebration. The historical file will be completely available to anyone designated by the Council or the Anniversary Committee to undertake this task.

JOHN A. STEVENSON, *Chairman*

Report of the Committee on Extension. The entire committee has not met formally this year; however, several members have met informally and contacts via correspondence have been maintained. The committee sponsored a breakfast symposium on the "Present Status and Outlook for Antibiotics in Plant Disease Control" as part of the A.P.S. Meetings in Atlanta. This was well attended by 60 men, most of whom participated in one way or another. A state-by-state report of antibiotic experiments and recommendations was useful to all. A mimeographed summary was prepared and transmitted to all registrants and other interested persons.

Other efforts and objectives of the 1956 committee include 1) strengthening the national Extension pathology program by encouraging all states to employ at least one full-time Extension pathologist; 2) maintaining close working relations with the fungicide industry by mutual exchange of toxicity data, labels, recommendations, and help with the Miller Amendment; 3) compiling color slide sets of plant diseases; 4) evaluating new methods of visualizing plant pathology via films, television, color bulletins, etc.; and 5) improving the undergraduate training of county agents in college.

The committee will sponsor an Extension Workers' breakfast at Cincinnati at which the above problems will be considered and discussed.

ARDEN F. SHERF, *Chairman*

Report of the Committee on Seed and Plant Material Certification. The committee program that was described (PDR 38: 521-528, 1954) has been in progress since 1950. A considerable amount of information has been assembled that was not previously available. In 1956, methods for production of disease-free material of certain ornamental plants were described by the ornamental crop subcommittee (PDR Supplement 238, pp. 57-95), and a preliminary report was published explaining services available from the Plant Quarantine Branch, U.S.D.A. for assistance to American exporters in meeting foreign phytosanitary requirements. Other projects from which reports can be expected are on certification of southern crops and development of virus-free stone fruit crops. No work is in progress on potatoes or vegetable crops. Further study is needed on disease problems in certification of forage crops.

JOHN R. HARDISON, *Chairman*

Report of the Committee on Resolutions. Be it re-

solved that the American Phytopathological Society in annual assembly at Cincinnati, Ohio, December 8, 1956, do hereby recognize with gratitude and convey appropriate expressions of appreciation to 1) The Committee on Local Arrangements for individual member efforts in providing adequate facilities for this convention (C. W. Ellett, Chr., C. C. Allison, Stephen Diachun, R. J. Green, Jr., Allen W. Troxel, and Lansing E. Williams); 2) the Officers and Council members for their efficient organization and administration of the many details necessary to a smooth functioning of the affairs of the Society; 3) the many members (those unsung heroes) of the various standing and special committees for their unstinting services on behalf of the Society; 4) and to Don Stewart, Bob Bills, and R. O. Bulger who provided the exhibit on barberry and stem rust located along the wall opposite the registration desk.

C. S. HOLTON, *Chairman*

Report of Elections, Appointments, and Other Business Actions. The Council counted the ballots of the annual election of new officers. The following were declared elected: *President Elect*, Paul R. Miller; *Vice President*, Glenn S. Pound; *Councilor-at-Large*, Roy A. Young.

Upon recommendation of the Council, the Society approved the appointment of W. B. Hewitt as Secretary for a 3-year term, of D. A. Roberts as Advertising Manager of PHYTOPATHOLOGY, and of M. W. Allen, C. E. Cox, W. L. Smith, Jr., Russell Stevens, and H. E. Wheeler as Editors of PHYTOPATHOLOGY, each for a 3-year term beginning January 1, 1957 (See change in Standing Rules below).

Representatives and Committees as listed above were appointed and approved.

The Society approved recommendations of the Council as follows:

1) That item (a) of Standing Rule 2 which reads "*Editorial Board.* The editorial policy of the official journal, PHYTOPATHOLOGY, shall be vested in an Editorial Board, consisting of an Editor-in-Chief, three Editors, and twelve Associate Editors; the business control shall be vested in a Business Manager and an Advertising (or Assistant Business) Manager" be changed to read as follows: "*Editorial Board.* The editorial policy of the official journal, PHYTOPATHOLOGY, shall be vested in an Editorial Board consisting of an Editor-in-Chief and fifteen Editors; the business control shall be vested in a Business Manager and an Advertising (or Assistant Business) Manager."

That item (c) of Standing Rule 2 which reads "*Terms of Office.* The term of each member of the Board shall be three years, provided that the terms of the three Editors shall not be concurrent and that four Associate Editors shall be appointed each year. The Council is authorized to make any adjustments in these terms necessary to effect non-concurrence" be changed to read as follows: "*Terms of Office.* The term of each member of the Board shall be three years and the appointments, exclusive of the Editor-in-Chief, shall be staggered so that five members are appointed each year. The Council is authorized to make any adjustments in these terms of office necessary to effect non-concurrence."

2) That the Standing Committee on Donations and Legacies be discharged and that their program be transferred to another committee.

3) That the Special Committee on Nomenclature and Classification of Plant Viruses be discharged.

4) That the Special Committee on Publication of Special Material be discharged.

5) That a Standing Committee on Phytopathological Reviews be erected, the chairman of which is to be a member of the Editorial Board of PHYTOPATHOLOGY. The duties of this committee will be to solicit and do preliminary editing of review papers for PHYTOPATHOLOGY. Members of this committee are named above.

6) That a Standing Committee on Publication of Phytopathological Monographs be established. This committee is authorized to consider publication of a series of phytopathological monographs in a manner similar to that of Phytopathological Classics, to choose an author or authors and subject or subjects, and to be authorized to mention to a prospective author a figure up to $2,000 as a Grant-in-Aid and report back to the Council any specific proposal or proposals for final clearance. Whereas publication of such a series is to be self supporting through sales, the initial publication costs will be underwritten by the Society. Members of this committee are named above.

7) That $75 be appropriated as a contribution to the Policy Committee for Scientific Agricultural Societies and to be given to the Policy Committee only if their budget is not raised by other means than by donations of affiliated societies.

8) That the contribution to the A.I.B.S. for 1957 be on the basis of one dollar per member (as done in 1956) rather than the $1000 maximum required of member Societies. This would mean a payment for 1957 of $1817.

9) That the $1500 budget provided for the Standing Committee on Public Relations for the past few years be continued for 1957.

10) That the $5 membership now offered to students be extended to men called into military service on a noncommissioned basis.

11) That a new membership directory be prepared to be issued in concurrence with our 50th Anniversary celebration.

12) That an additional $7500 be appropriated as a fund for use in observance of the Society's fiftieth anniversary. This makes a total appropriation of $15,000 for this event.

Future Meetings. The 1957 annual meeting will be held on the campus of Stanford University, August 26, 27, and 28, in conjunction with the A.I.B.S. The 1958 meeting will be our Golden Anniversary and will be held with A.I.B.S. on the campus of the University of Indiana, August 24-28.